Genotoxic-activated G_2-M checkpoint exit is dependent on CDC25B phosphatase expression

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Abstract

Cell cycle arrest at the G_2-M checkpoint is an essential feature of the mechanisms that preserve genomic integrity. CDC25 phosphatases control cell cycle progression by dephosphorylating and activating cyclin-dependent kinase/cyclin complexes. Their activities are, therefore, tightly regulated to modulate cell cycle arrest in response to DNA damage exposure. Here, we report that overexpression of CDC25B affects viability, reduces clonogenic efficiency, and increases sensitivity of cancer cells to a genotoxic agent. We show that ectopic expression of CDC25B results in bypass of a genotoxic-induced G_2-M checkpoint. In addition, cancer cells constitutively expressing high level of CDC25B are shown to be prone to exit prematurely from the G_2-M checkpoint arrest and to enter mitosis. Finally, we show that this exit is dependent on CDC25B expression. Together with previous results, our data strongly support a model in which CDC25B is the key phosphatase that controls entry into mitosis after DNA damage, thus emphasizing the relevance of its overexpression in many human tumors. [Mol Cancer Ther 2006;5(6):1446–51]

Introduction

The CDC25 phosphatases are major players in the control of cell proliferation. They dephosphorylate and activate cyclin-dependent kinases (CDK) at critical stages during the cell cycle. There are three members in the CDC25 family in humans (CDC25A, CDC25B, and CDC25C), which were initially reported to be involved in the control of the G_1-S transition and the progression through late G_2 and mitosis, respectively. However, a recent body of evidence indicate that they probably all participate in the control of entry into and progression through mitosis (1). As first shown by the pioneering work done in yeast (2), CDC25 activity is required to control the G_2-M transition, a major cell cycle checkpoint where the genomic integrity is verified before a cell can proceed to divide its genome into two daughter cells (3). In response to DNA injury, this G_2-M checkpoint becomes activated, leading to cell cycle arrest, which is maintained until the damage has been repaired or the cell undergoes apoptosis.

A number of studies dedicated to characterizing of the molecular pathways involved in this response have been conducted over the last few years. The emerging picture indicates that CDC25 phosphatases are major targets of the kinase cascades, involving the ATM/ATR and the CHK1-CHK2 kinases that are activated by the presence of DNA damage (4). CDC25A seems to be phosphorylated by the CHK1 kinase, which leads to its proteasome-dependent degradation (5, 6). Phosphorylation of CDC25A by CHK1 also creates a 14-3-3 binding site that specifically inhibits its ability to interact with CDK1-cyclin B (7). CDC25C phosphorylation by CHK1 similarly generates a 14-3-3 binding site to sequesters CDC25C in the cytoplasm and blocks its interaction with CDK1-cyclin B (8, 9). Evidence from our laboratory indicate that CHK1 also phosphorylate and inactivate CDC25B upon G_2-M checkpoint activation (10). Overall, the exact mechanisms by which all three CDC25 phosphatases activities are shutdown upon checkpoint activation are still not totally deciphered.

The mechanisms by which cells are able to restart the cell cycle and to enter mitosis is another issue that remains to be resolved. However, it was recently proposed that the CDC25B phosphatase but not CDC25A or CDC25C is an essential component of the machinery that enables a cell to resume cell cycle after DNA damage–induced cell cycle arrest (11). This ability to exit the G_2-M checkpoint was also shown to be dependent on PLK1-dependent degradation of WEE1, the kinase that phosphorylates and inhibits CDKs. This probably then allows CDC25B to more efficiently activate CDK1-cyclin B (11, 12).

Thus, altering the tight regulation of these mechanisms could be responsible for the increased genomic instability observed in cancer cells. The reported elevated expression of PLK1 and CDC25B in a number of human tumors and their correlation with high-grade and/or poor prognosis strongly argue in favor of this hypothesis (13, 14). It is also likely to be highly relevant to take into account the

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modification of the expression of key regulators of the G_2-M checkpoint recovery process, including CDC25B, in the selection and the adaptation of the chemotherapeutic strategy using genotoxic agents.

In this study, we have examined the importance of CDC25B expression level in G_2-M control and in the cellular response to genotoxic exposure. Our results confirm that CDC25B is a key regulator of checkpoint exit and support a model in which altered expression is responsible for premature checkpoint exit and increase sensitivity to genotoxic agents.

Materials and Methods

Cell Culture and Transfection

U2OS cells expressing Ha-CDC25B3 under tetracycline-regulated promoter (U2OS-CDC25B3) were grown and induced as previously described (15). Hep-2 cells (American Type Culture Collection, Rockville, MD) were maintained in MEM supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, and penicillin/streptomycin. MiaPaCa 2 (American Type Culture Collection) were maintained in supplemented with 10% fetal bovine serum and penicillin/streptomycin. All cell lines were maintained at 37°C in a 5% CO_2 atmosphere.

Small interfering RNA (siRNA) oligonucleotides corresponding to CDC25B and scramble sense strands were purchased from Dharmacon (Lafayette, CO). The vector producing short hairpin RNA (shRNA) CDC25B was constructed in the pRNATH1.1 GFP expressing vector (Genescript, Piscataway, NJ). The sequence targeting the human CDC25B were based on a 19-mer sequence previously described (11). Hep-2 cells were electrotransfected using the Amaxa nucleofector, with vector expressing siRNA alone or added of siRNA from Dharmacon to amplify the CDC25B extinction, as indicated in Fig. 4 legend.

Clonogenic and Poly(2-Hydroxyethyl Methacrylate) Assays

U2OS cells were seeded on plastic dishes (1,000 for 3.5 cm^2) in the presence or in absence of tetracycline and together with compounds at the indicated concentration. After 7 days, colonies were stained with crystal violet. Poly(2-hydroxyethyl methacrylate), from Sigma (St. Louis, MO), is an antiadhesive polymer used by Fukazawa et al. (16) to quantitate anchorage independent growth capacity in 96-well plates. Fifty microliters of a poly(2-hydroxyethyl methacrylate) solution at 5 mg/mL in ethanol were added into wells of 96-well plates and dried for 2 days. U2OS-CDC25B3 cells were seeded at 1,000 to 5,000 per well in DMEM with or without 2 μg/mL of tetracycline. This experiment was done with eight replica per concentration tested. Five days later, the extent of cell proliferation was evaluated by a colorimetric assay based on the cleavage of the tetrazolium salt WST1 by mitochondrial dehydrogenases in viable cells, leading to formazan formation (Roche Diagnostic, Meylan, France).

Flow Cytometry

Cells were harvested and fixed in ice-cold 70% ethanol, washed with PBS-1% bovine serum albumin, and permeabilized with 0.25% Triton X-100 for 10 minutes. Cells were stained with rabbit anti-phospho-histone H3 (Upstate Biotechnology, Lake Placid, NY) followed by anti-IgG Alexa 488. DNA was stained with propidium iodide (10 μg/mL) in the presence of RNase (10 μg/mL).

Cell cycle distribution and phospho-histone H3 positivity cells were determined using flow cytometric analyses (Cell Quest, Becton Dickinson, Mountain View, CA). To select the transfected cells, a paraformaldehyde fixation was done with the intrastain kit from DakoCytomation (Carpinteria, CA), in the place of the ethanol fixation, and then the cells were stained with anti-phospho H3 as above.

Immunofluorescence

Cells cultivated onto glass coverslips were fixed 15 minutes in 3.7% formaldehyde and permeabilized for 15 minutes in 0.5% Triton X-100 in PBS. Detection was done using anti-phospho-histone *γH2AX* (Ser139; Upstate Biotechnology) diluted 500-fold in blocking buffer (0.1% Tween 20/3% bovine serum albumin in PBS) followed by incubation with a goat anti mouse Alexa 594 (Molecular Probes, Eugene, OR) at a dilution of 1:400 in blocking buffer. Images were acquired using a DM6000 microscope (Leica, Wetzlar, Germany).

Western Blot

Cells were lysed as previously described (10), electrophoresed on a 4% to 12% SDS gradient gel (Invitrogen, Carlsbad, CA) and analyzed by Western blotting. CDC25B rabbit polyclonal antibody (C-20) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-actin monoclonal antibody was from Chemicon (Temecula, CA).

Results

Overexpression of CDC25B Affects Cell Viability, Reduces Clonogenic Efficiency, and Increases Sensitivity to Genotoxic Treatment

To examine the effect of CDC25B overexpression, we made use of a U2OS cell line (U2OS-CDC25B3) that conditionally expresses the CDC25B phosphatase under the control of the tetracycline repressible promoter (17). We first monitored in the absence of any DNA-damaging agent the clonogenic potential of U2OS-CDC25B3 cells in the presence or absence of tetracycline (i.e., overexpressing or not the CDC25B phosphatase, respectively). As shown in Fig. 1A, the clonogenic potential of the cells on plastic was reduced by about 80% upon continuous expression of CDC25B over 7 days. Similarly, seeding U2OS-CDC25B3 cells at increasing density on poly(2-hydroxyethyl methacrylate)–coated wells strongly inhibited their capacity for anchorage-independent growth (Fig. 1B). These results confirmed that constitutive expression of CDC25B is detrimental to cell division and might result in loss of viability.

Taking into account these observations, we next investigated whether overexpression of CDC25B could also affect the sensitivity of U2OS cells to treatment with the genotoxic agent etoposide (VP-16). Cells were seeded

on plastic dishes, and CDC25B expression was induced (ON) or not (OFF) to determine their clonogenic potential in the continuous presence of increasing concentration of VP-16. The results of three independent IC_{50} determinations are reported in Fig. 1C. These data indicated that the IC_{50} for VP-16 was about 30% lower when CDC25B was expressed.

Bypass of a Genotoxic-Induced G_2-M Checkpoint Arrest by Ectopic Expression of CDC25B

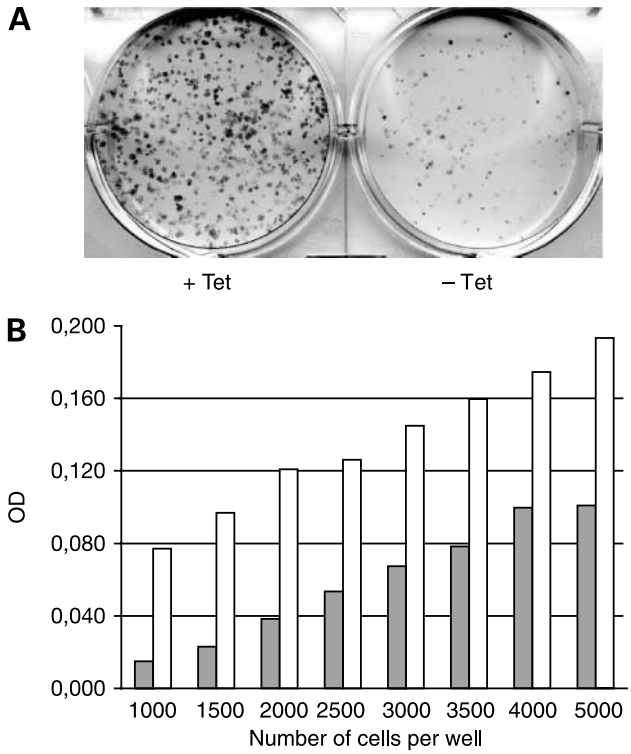
The data presented in Fig. 1 suggested that increased expression of CDC25B may induce premature and unscheduled G_2-M transition to overcome checkpoint activation, or speed up exit from the arrest and thus induce unscheduled entry into mitosis, resulting in loss of cell viability. To validate this hypothesis, we tested the effect of ectopic expression of CDC25B on the cellular response to G_2-M checkpoint activation by DNA-damaging agents. This goal was achieved using U2OS-CDC25B3 cells in an assay similar to that developed by Roberge's laboratory (18). Cells overexpressing or not CDC25B were treated with 40 μmol/L VP-16 for 1 hour to induce DNA damage and then grown for an additional 24 hours in the presence of nocodazole. Thus, if the cells were able to exit the DNA damage–dependent G_2-M arrest, they were subsequently trapped by the presence of nocodazole in abnormal mitosis at the spindle assembly checkpoint. The accumulation of cells in mitosis was, therefore, a direct indicator of checkpoint bypass efficiency. We monitored the cell cycle distribution by flow cytometry after propidium iodide staining and immunolabeling with antibodies against phosphorylated histone H3. As shown in Fig. 2A, cells exposed to VP-16 in the absence of CDC25B expression (promoter OFF) accumulated mostly in G_2. In contrast, CDC25B expression had a mitotic-inducing effect that probably resulted from a bypass of the G_2-M checkpoint and the subsequent accumulation of cells in the nocodazole-dependent ''mitotic trap.'' In the experiment presented here, about 20% of the cells had bypassed the G_2-M checkpoint, but this effect could be enhanced, depending on the duration of the CDC25B expression (data not shown). A similar experiment was achieved by treatment with UCN-01, a potent checkpoint-1 kinase inhibitor (19), which lead to >50 % of the cells bypassing the G_2-M checkpoint arrest and accumulating in mitosis (data not shown).

To confirm that the observed checkpoint bypassing effect was in fact dependent on the CDC25B phosphatase activity, we did a similar experiment in which after genotoxic injury, the cells were also treated with BN82685, a chemical inhibitor of CDC25 phosphatase (20). We found that induced U2OS-CDC25B3 cells treated with 500 nmol/L BN82685 were unable to bypass the VP-16-induced cell cycle checkpoint arrest and therefore remained in G_2-M (Fig. 2B). Furthermore, the degree of G_2-M checkpoint bypass was directly dependent on the concentration of BN82685 used in the experiment (Fig. 2B).

These data confirmed that ectopic expression of CDC25B is sufficient to induce the bypass of a DNA damage–activated checkpoint as already reported (21) and further showed that use of a specific CDC25 inhibitory compound can revert this effect.

Cells Expressing High Level of CDC25B Are Prone to Bypass the G_2-M Checkpoint

We next examined the efficiency of the G_2-M checkpoint in cancer cells that endogenously express higher levels of CDC25B than U2OS cells. As shown by Western blot analysis done on total proteins extracts (Fig. 3A), the level of CDC25B was found to be lower in U2OS cells compared with other cell lines, such as MiaPaCa or



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CDC25B	Etoposide IC_{50} (nM) Exp #1	Exp #2	Exp#3
ON	40	37	55
OFF	61	48	79

Figure 1. Overexpression of CDC25B affects cell viability, reduces clonogenic efficiency, and increases sensitivity to a genotoxic treatment. **A**, U2OS-CDC25B3 cells (17) were seeded on plastic dishes and grown in the presence or absence of tetracycline. After 7 d, the plates were stained with crystal violet to reveal growing individual clones. Representative of results obtained from three separate experiments. **B**, U2OS-CDC25B3 cells were seeded at increasing density on poly(2-hydroxyethyl methacrylate) – coated wells in the presence (*open columns*) or in the absence of tetracycline (*gray columns*). Cell proliferation was monitored by using the WST1 colorimetric cell proliferation assay. **C**, induced or uninduced U2OS-CDC25B3 cells were seeded on plastic dishes in the continuous presence of increasing concentration of VP-16. After 7 d, the plates were stained with crystal violet, and IC_{50} values were determined by counting the individual colonies. Triplicate calculations were done for each concentration used. Results of three independent experiments.

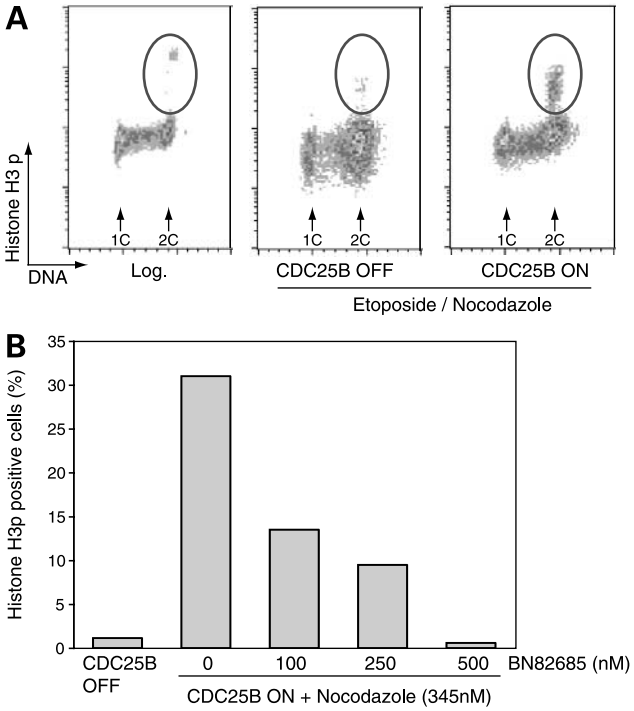

Figure 2. Expression of CDC25B in U2OS osteosarcoma cells results in bypass of the G_2-M checkpoint. U2OS-CDC25B3 cells (17) were grown in the presence or absence of tetracycline for 24 h and then treated for 1 h with 40 μmol/L VP-16. The cells were washed and further incubated in the presence of 345 nmol/L nocodazole alone (**A**) or together with the BN82685 CDC25 phosphatase inhibitor (**B**). **A**, flow cytometry analyses of DNA content and histone H3 phosphorylation. Positive histone H3 phosphorylated cells are indicated with a red circle. Analysis was done after gating for live cells. Arrowheads indicate the cell populations with G_1 and G_2 DNA contents. Log are untreated U2OS-CDC25B3 grown in the presence of tetracycline. **B**, determination of the percentage of induced U2OS-CDC25B3 cells (CDC25B ON) with positive histone H3 phosphorylation in the presence of increasing BN82685 concentration. Cells not expressing CDC25B (CDC25B OFF) are presented as a control population not treated with nocodazole.

Hep2 cells. The identity of the protein detected by Western blot analyses using a specific antibody was confirmed using CDC25B-targeting shRNA vector transfection, which almost completely abolished the CDC25B signal (see Fig. 3A, *right*).

Having shown (see Fig. 2A) that the U2OS cell line expressing endogenously low levels of CDC25B (promoter OFF) efficiently stopped the cell cycle at the G_2-M checkpoint after treatment with a genotoxic agent, we examined the effect of doxorubicin treatment on Hep2 and MiaPaCa cells using the mitotic trap assay. The concentration of doxorubicin use in the assay were sufficient to induce DNA damage as indicated by immunofluorescence staining with antibodies against histone H2AX (Fig. 3B). As shown in Fig. 3C, arrest at the G_2-M checkpoint upon DNA injury and subsequent entry into mitosis was dependent on the concentration of doxorubicin used. At the low concentration of 52 and 86 nmol/L for MiaPaCa and Hep2, respectively (Fig. 3C), cells did not arrest at the G_2-M checkpoint while they were unable to progress into mitosis when treated with higher concentration. The ability of these cells to resume the cell cycle and to enter mitosis after DNA damage–induced G_2-M arrest was found to be dependent on CDC25 activity, being inhibited

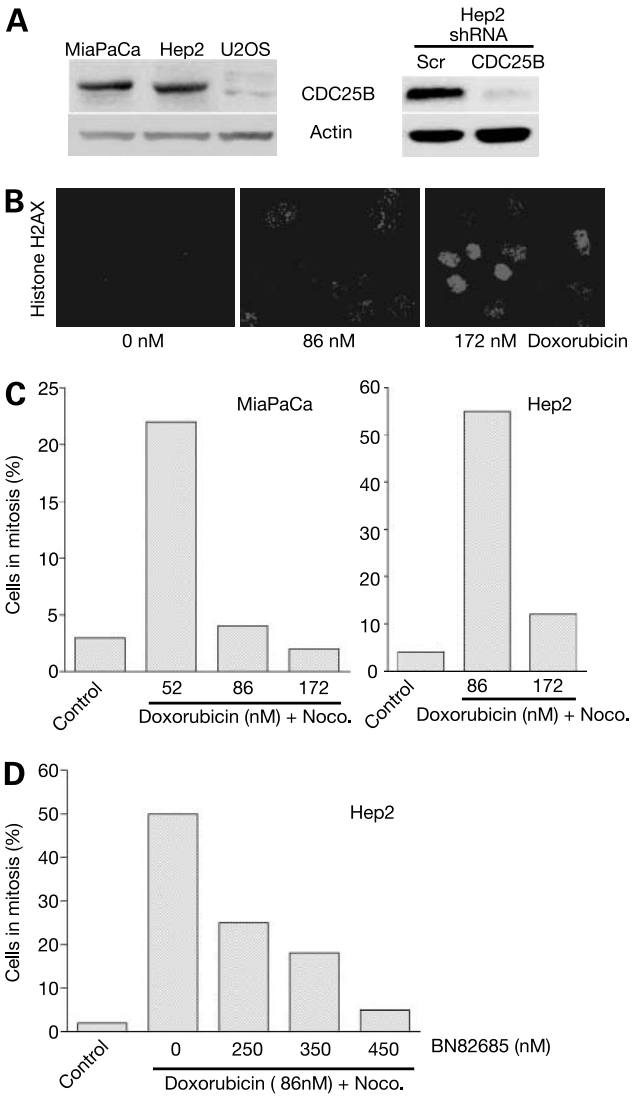

Figure 3. Cells expressing high level of CDC25B are prone to bypass the G_2-M checkpoint. **A**, *left,* Western blot analysis of CDC25B expression in U2OS, Hep2, and MiaPaCa cells using a polyclonal antibody raised against the COOH-terminal end of CDC25B. *Right,* Hep2 cells were transfected with control or specific shRNA for CDC25B. Transfected cells were selected with G418 (1 mg/mL) for 24 h after transfection. **B**, Hep2 cells were treated for 4 h with the indicated concentrations of doxorubucin then fixed and examined by immunofluorescence after staining with anti-histone H2AX antibodies to detect DNA damage. **C**, Hep2 and MiaPaCa cells were treated with increasing concentrations of doxorubicin during 4 h then with 345 nmol/L nocodazole alone for 17 h. The percentage of cells that had overcome the G_2-M block and were then trapped in mitosis was determined by flow cytometry after Histone H3p determination (as in Fig. 2). **D**, Hep2 cells were treated as in **C** with 86 nmol/L doxorubicin then nocodazole together with increasing concentrations of the CDC25 inhibitor BN82685.

in a concentration-dependent manner by the BN82685 compound (Fig. 3D). Inhibition of CDC25 activities, therefore, resulted in the inability of G_2-M-arrested cells to overcome cell cycle arrest induced by DNA damage and to enter mitosis.

G_2-M Checkpoint Exit in Tumor Cells Is Dependent on CDC25B Expression

Although chemical inhibitors, such as BN82685, are highly selective for CDC25 phosphatases (20), they have a broad activity against all three CDC25 family members. Thus, to show that checkpoint exit efficiency was a CDC25B-specific feature, we reduced the expression of CDC25B in Hep2 cells using a combined shRNA/siRNA strategy and examined their response to DNA-damaging agents. Cells were transfected with shRNA/siRNA (see Materials and Methods) and then subsequently treated after 48 hours with doxorubicin and nocodazole. Quantification of entry into mitosis was again assessed by flow cytometry analyses after histone H3p labeling. As shown in Fig. 4A, under these conditions, expression of CDC25B was largely reduced. As presented in Fig. 4B, a significant fraction of cells transfected with scrambled siRNA/shRNA entered mitosis and were trapped by nocodazole as described above. In contrast, when the cells were transfected with the CDC25B siRNA/shRNA, entry into mitosis was consistently inhibited, although not completely. The individual results from five independent experiments are presented. Although the percentage of mitotic cells varied from experiment to experiment, we consistently observed a decrease in the ability of the cells to enter mitosis following transfection with CDC25B siRNA/shRNA. These results indicate that CDC25B expression is one of the key variables required for entry into mitosis after activation of the G_2-M checkpoint.

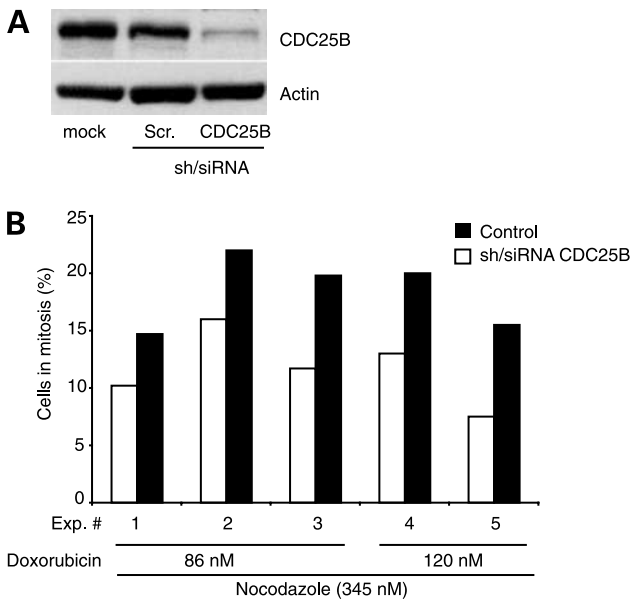

Figure 4. G_2-M checkpoint exit in tumor cells is dependent on CDC25B expression. Hep2 cells were treated for 48 h with 50 or 70 ng/mL doxorubicin and 200 ng/mL nocodazole, and the percentage of cells trapped in mitosis was determined. Before doxorubicin treatment, the cells were transfected with pRNAth 1.1 vector empty or allowing the expression of CDC25B shRNA (experiments 1, 2, and 4), or with the same plasmid together with scrambled or specific CDC25B siRNA from Dharmacon (experiments 3 and 5). **A**, Western blot analysis of the CDC25B expression in Hep2 cells either mock-transfected or transfected with scramble or shRNA for CDC25B. **B**, quantification of the cells in mitosis by flow cytometry after histone H3p labeling.

Discussion

CDC25 phosphatases are key actors of the control of entry and progression into mitosis. However, the contribution of each one of the three human CDC25 phosphatases in the activation of CDK-cyclin complexes to control entry into mitosis after a DNA damage checkpoint arrest in G_2 remains currently unclear. It has recently been proposed that CDC25B is required for an efficient exit of cells from a G_2-M checkpoint activated by DNA-damaging agents (11). The Plk1 kinase is also required in this process, probably by participating in the control of the degradation of the Wee1 inhibitory kinase (12). These results suggested that the activity of CDC25B could be an important variable in the cellular response to DNA damage through the modulation of the cell's ability to escape G_2-M arrest and enter mitosis when injuries have been repaired. However, an increase in the endogenous expression of CDC25B, as has been observed in a number of human tumors, might also be responsible for an alteration in the physiologic response to DNA damage, thus leading to inappropriate behaviour of the cells. In the present study, we have tested this hypothesis using cancer cell lines in which CDC25B was either overexpressed or reduced either pharmacologically or using an interference RNA strategy.

In the first place, we have been able to show in a clonogenic assay that elevated expression of CDC25B in osteosarcoma U2OS cells results in decreased cell viability and is sufficient to increase the cytotoxicity induced by genotoxic agents, such as VP-16.

We further showed that overexpression of CDC25B in U2OS cells leads to an accelerated exit of cells from the G_2-M arrest after checkpoint activation. This acceleration is dependent on the catalytic activity of the CDC25 phosphatase, as it can be reverted with the use of a specific pharmacologic inhibitor of CDC25. Such a G_2-M checkpoint bypass was not observed upon conditional overexpression of CDC25C in U2OS cells (data not shown; as this was already suggested from work of Medema's group, indicating that CDC25C was not required for exit from a G_2-M arrest after DNA damage; ref. 11).

Exit or bypass from a G_2-M arrest was examined in cancer cells that inherently express higher levels of CDC25B than U2OS cells, following exposure to genotoxic agents. We found this response to vary depending on the cell type and the concentration of drug used. However, in every case, the ability of the cell to bypass G_2-M could be abolished by pharmacologic inhibitors of CDC25. To prove

that this was a specific effect of eliminating CDC25B activity, we showed that this response was reduced upon partial invalidation of CDC25B expression using the interference RNA technology.

We have also shown that in tumor cells, such as osteosarcoma U2OS cells in which the level of CDC25B was found to be low, the ability to escape a G_2-M arrest induced by low doses of genotoxic agent was reduced or abolished.

This work shows that the expression of CDC25B is an important variable conditioning the ability of the cells to enter mitosis when they have been subjected to genotoxic injury. The observed increase in cytotoxicity in response to genotoxic treatment in cells expressing high levels of CDC25B suggests that it may be essential to take into account this expression level in the therapeutic decision. As suggested by our data, the activity and expression levels of CDC25B could affect the efficiency of genotoxic therapy by influencing the rate at which cells reinitiate mitosis after damage or escape the G_2-M checkpoint. Moreover the propagation of defects in the genome, caused by the inappropriate entry into mitosis, to daughter cells may contribute to tumor growth. However, further studies in which the expression of CDC25B in tumors is compared with the outcome of chemotherapy will need to be done to validate this hypothesis.

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References

1. Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol 2006;18:185 – 91.

2. Russell P, Nurse P. cdc25$^+$ functions as an inducer in the mitotic control of fission yeast. Cell 1986;45:145 – 53.

3. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science 1996;274:1664 – 72.

4. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev 2001;15:2177 – 96.

5. Mailand N, Podtelejnikov AV, Groth A, Mann M, Bartek J, Lukas J. Regulation of G(2)/M events by Cdc25A through phosphorylation-dependent modulation of its stability. EMBO J 2002;21:5911 – 20.

6. Xiao Z, Chen Z, Gunasekera AH, et al. Chk1 mediates S and G_2 arrests through Cdc25A degradation in response to DNA-damaging agents. J Biol Chem 2003;278:21767 – 73.

7. Chen MS, Ryan CE, Piwnica-Worms H. Chk1 kinase negatively regulates mitotic function of Cdc25A phosphatase through 14 – 3-3 binding. Mol Cell Biol 2003;23:7488 – 97.

8. Sanchez Y, Wong C, Thoma RS, et al. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 1997;277:1497 – 501.

9. Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G_2 checkpoint control: regulation of 14.3.3 protein binding by phosphorylation of CDC25C on Serine-216. Science 1997;277: 1501 – 5.

10. Cazales M, Schmitt E, Montembault E, Dozier C, Prigent C, Ducommun B. CDC25B phosphorylation by Aurora-A occurs at the G_2/M transition and is inhibited by DNA damage. Cell Cycle 2005;4:1233 – 8.

11. van Vugt MA, Bras A, Medema RH. Polo-like kinase-1 controls recovery from a G_2 DNA damage-induced arrest in mammalian cells. Mol Cell 2004;15:799 – 811.

12. Watanabe N, Arai H, Iwasaki J, et al. Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. Proc Natl Acad Sci U S A 2005;102:11663 – 8.

13. Kristjansdottir K, Rudolph J. Cdc25 phosphatases and cancer. Chem Biol 2004;11:1043 – 51.

14. van Vugt MA, Medema RH. Getting in and out of mitosis with Polo-like kinase-1. Oncogene 2005;24:2844 – 59.

15. Davezac N, Baldin V, Gabrielli B, et al. Regulation of CDC25B phosphatases subcellular localization. Oncogene 2000;19:2179 – 85.

16. Fukazawa H, Mizuno S, Uehara Y. A microplate assay for quantitation of anchorage-independent growth of transformed cells. Anal Biochem 1995;228:83 – 90.

17. Theis-Febvre N, Filhol O, Froment C, et al. Protein kinase CK2 regulates CDC25B phosphatase activity. Oncogene 2003;22:220 – 32.

18. Roberge M, Berlinck RG, Xu L, et al. High-throughput assay for G_2 checkpoint inhibitors and identification of the structurally novel compound isogranulatimide. Cancer Res 1998;58:5701 – 6.

19. Graves PR, Yu L, Schwarz JK, et al. The chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01 [in process citation]. J Biol Chem 2000;275:5600 – 5.

20. Brezak MC, Quaranta M, Contour-Galcera MO, et al. Inhibition of human tumor cell growth *in vivo* by an orally bioavailable inhibitor of CDC25 phosphatases. Mol Cancer Ther 2005;4:1378 – 87.

21. Karlsson C, Katich S, Hagting A, Hoffmann I, Pines J. Cdc25B and Cdc25C differ markedly in their properties as initiators of mitosis. J Cell Biol 1999;146:573 – 84.

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