Eukaryotic cells have developed a network of checkpoints to ensure their survival and propagation of accurate copies of the genome to the next generation when suffering from a variety of stress. Unlike classic signal transduction pathways that respond to one or a few stimuli, checkpoints can be triggered by a broad range of intrinsically or extrinsically damaged DNA (1, 2). The activated cell cycle checkpoint machinery coordinates cell cycle progression with DNA repair, chromatin remodeling, transcriptional activation, and other metabolic adjustments or even cell death (1). The biological signaling cascades can trigger several different outcomes: (a) reentry into the cell cycle with repaired DNA; (b) cellular senescence when the damage is beyond repair; (c) apoptosis to eliminate cells that are likely to become cancerous (3); and (d) checkpoint adaptation (cell division with unrepaired DNA lesions; ref. 4).

Cdc25A, one of the phosphatases of the Cdc25 family (Cdc25A, Cdc25B, and Cdc25C), is an essential regulator of the cell cycle machinery, functioning as a positive regulator by activating cyclin-dependent kinases (CDK; ref. 5). Cdc25A regulates both G1-S and G2-M cell cycle transitions (5). In response to DNA damage, for example, due to ionizing or UV radiation, Cdc25A is rapidly degraded. Consequently, Cdc25A is not able to dephosphorylate and thereby activate CDKs, leading to a delay in cell cycle progression (6). Cdc25A degradation can also be activated when anticancer drugs are used to induce DNA damage; for example, topoisomerase inhibitors induce rapid Cdc25A turnover, whereas the alkylating agent cisplatin causes delayed Cdc25A degradation (7). Moreover, Cdc25A is a proto-oncogene, overexpression of which acts in synergy with H-Ras expression or loss of pRb expression to transform fibroblasts in a mouse cancer model (8). Overexpression of Cdc25A has been found in at least 10 types of human cancers, including liver, breast, head and neck, lung, esophageal, and colorectal cancers (9). High Cdc25A expression has been shown to be associated with aggressive tumors and poor clinical outcomes (9).

Abstract

Purpose: Most studies on pathogenesis of tumor metastasis focus on cell adhesion and migration. Little is understood of how cell cycle pathways critically affect cell fate of metastatic cells and their sensitivity to anticancer drugs. In this study, we investigated cell cycle checkpoint progression and regulation in the presence of cisplatin in metastatic hepatocellular carcinoma (HCC) cells.

Experimental Design: Cisplatin-mediated cell cycle progression and Polo-like kinase 1 (Plk1)-Cdc25A pathway were compared between metastatic and nonmetastatic HCC cells by flow cytometry, Western blots, and reverse transcription-PCR. Cdc25A expression in clinical HCC samples was detected using immunohistochemistry and its association with clinical HCC metastasis was analyzed.

Results: Cisplatin induced degradation of Cdc25A in nonmetastatic HCC cells but not in metastatic HCC cells. Hence, metastatic HCC cells showed defective S-M cell cycle phase arrest and continued to enter mitosis. Tumor expression of Cdc25A was strongly associated with metastatic diseases in HCC patients, and elevated Cdc25A expression significantly correlated with HCC tumor-node-metastasis staging and venous invasion. Metastatic HCC cells did not show down-regulation of Plk1 that was normally induced by DNA damage. Blockage of Plk1 expression in metastatic HCC cells initiated Cdc25A degradation in response to DNA damage, suggesting that Plk1 could be an upstream regulator of Cdc25A. Deregulated Plk1-Cdc25A pathway in metastatic HCC cells and primary tumors did not result in drug-induced mitotic catastrophe but rather in accumulation of damaged DNA due to checkpoint adaptation.

Conclusions: Metastatic HCC cells showed a defective S-M checkpoint following cisplatin treatment and potential aberrant checkpoint adaptation, which might result from deregulation of Plk1-Cdc25A pathway.
**Translational Relevance**

Overexpression of Cdc25A has been found in at least 10 types of human cancers and shown to be associated with aggressive tumors and poor clinical outcomes. The present study identified that high expression of Cdc25A is strongly associated with metastatic diseases in HCC patients. It also identified the aberrant Plk1-Cdc25A pathway in metastatic HCC cells and in HCC primary tumors, resulting in defective S-M checkpoint, and accumulation of damaged DNA due to checkpoint adaptation. We first identified that Plk1 is a potential upstream regulator of Cdc25A, and this can be translated from HCC cell lines to HCC patient tumors. Plk1 has received increased attention as a putative cancer treatment target after a small-molecule inhibitor of Plk1 showed significant antitumor activity in vivo. The deregulated Plk1-Cdc25A pathway in metastatic HCC provides the molecular basis for using Plk1 inhibitors as therapeutics to treat metastatic HCC.

Although the ATM/ATR-Chk1/Chk2-Cdc25 pathway is a classic one for responding to DNA damage (10), other upstream regulators of Cdc25 proteins, such as Polo-like kinases (Plk), are also important cell cycle regulators, functioning in centrosome maturation, spindle formation, mitotic entry, and cytokinesis (11). When responding to DNA damage, Plk1 triggers cell cycle arrest in the G2 and M phases (12), and Plk3 is activated in response to genotoxic stress (13, 14). Plk3 can phosphorylate Cdc25A and Cdc25C, which may regulate the stability of Cdc25A and promote nuclear accumulation of Cdc25C (11). Less is known about the role of Plk1 in regulating Cdc25 at the cell cycle checkpoint. Recently, Plk1 has been shown to regulate recovery after DNA damage-induced checkpoint arrest via a mechanism dependent on Plk1 and Cdc25B (15). It has also been shown to regulate checkpoint adaptation in mammalian cells via an unknown pathway (15, 16).

Most studies regarding pathogenesis of tumor metastasis focus on cell adhesion and migration. However, regulation of the cell cycle and cellular proliferation is also critical for determining the fate of metastatic cells and their sensitivity to anticancer drugs. In the present study, we found that cisplatin-mediated degradation of Cdc25A was abnormal in metastatic hepatocellular carcinoma (HCC) cells, and this correlated with a defective S-M phase delay. Our results further suggested the cause to be an aberrant Plk1-Cdc25A regulatory pathway. Overexpressed Cdc25A in tumor of HCC patients was strongly associated with tumor metastases, which supports our results in cell culture. Finally, we explored the biological consequences of a deregulated Plk1-Cdc25A interaction, which did not result in mitotic catastrophe but instead led to cell division with accumulated DNA damage lesion due to checkpoint adaptation.

**Materials and Methods**

Cell culture and drug treatment. Nonmetastatic HCC cell lines Hep3B, PLC (American Type Culture Collection), and H2P (17) and metastatic HCC lines MHCC97L, MHCC97H (18, 19), and H2M (17) were cultured in DMEM (Invitrogen/Life Technologies) containing 10% fetal bovine serum (Invitrogen) plus streptomycin and penicillin (Sigma). For each experiment, cells were seeded overnight before treatment with 1 μg/mL cisplatin (Mayne Pharma).

**Cell cycle analysis.** For measurement of S phase populations, cells were incubated in 30 μmol/L bromodeoxyuridine (Sigma) for 20 min before harvesting and fixing in 80% cold ethanol. The bromodeoxyuridine content was determined by reaction with a FITC-conjugated anti-bromodeoxyuridine antibody (Sigma), according to the manufacturer's instructions, plus propidium iodide-RNase (Sigma) counterstaining to determine DNA content. Mitotic cells were determined by reaction with anti-phospho-histone H3 (Ser10) antibody (Upstate). Samples were analyzed by FACScan (BD Biosciences) using the CellQuest software.

**Antibodies.** Anti-Cdc25A (sc-F6 and sc-97) was from Santa Cruz Biotechnology. Antibodies against phospho-Chk1 (Ser345), phospho-Chk2 (Thr389), and phospho-H2AX (Ser139) were from Cell Signaling. Antibodies to Plk1 and Plk3 were from Zymed and BD Biosciences.

**Western blots.** Total protein lysates (10-30 μg) were loaded and separated on a 10% or 12% SDS-PAGE gel and then transferred to a polyvinylidene difluoride membrane. Membranes were incubated with primary antibody overnight at 4°C and horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature.

**Immunofluorescence and immunohistochemistry.** H2P, H2M, PLC, and 97L cells were seeded over 40 h. Cells were fixed in 4% paraformaldehyde, permeabilized with 0.25% Triton X-100 in PBS, blocked with 10% goat serum and 1% bovine serum albumin in PBS, and incubated with antibody overnight at 4°C. Cells were then incubated with FITC-conjugated secondary antibody (Sigma) for 1 h and counterstained with 4',6-diamidino-2-phenylindole. Deparaffinized sections were boiled in a microwave for 10 min in citrate buffer for antigen unmasking. After blocking with 1% H2O2 and 10% goat serum, the sections were incubated with primary antibody overnight at 4°C. Polymer horseradish peroxidase-conjugated secondary antibody (DakoCytomation) was used to visualize the signal. The sections were counterstained with hematoxylin. Signal intensities were classified into four categories (20) according to the amount of positive cell staining as judged by two independent reviewers: -, <10%; +, 10-25%; ++, 25-75%; and +++, >75%.

**Reverse transcription-PCR.** Total RNA isolation, reverse transcription, and PCR were carried out as described previously (21). PCR primers were forward 5'-CGAGAAGACGGTGAGGTGGATG-3' and reverse 5'-AGAGGTAGCAGAGGCTGGTCTTAC-3' for Plk1 and forward 5'-GGAGCAAGAAGAAGACCA-3' and reverse 5'-AGGCAGGCTTATGAGAACCCT-3' for Plk3. PCR was carried out for 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The PCR products were run through a 1.5% agarose gel with ethidium bromide and visualized by transillumination.

**Small interfering RNA transfection.** Plk1 small interfering RNA was purchased from Santa Cruz Technology. Small interfering Plk1 and control RNA (Qiagen) at 50 to 100 pmol/L per 1 mL medium were transfected into cells using LipofectAMINE 2000 (Invitrogen) following the manufacturer's instructions. At 48 to 72 h after transfection, the cells were harvested for further experiments.

**Tumor specimens.** Tissue specimens of HCC were collected immediately after resection from 30 HCC patients ages 29 to 80 years and diagnosed to have stage I to IV pathologic tumor-node-metastasis disease (22). Tumor tissues derived from nonmetastatic (Hep3B and PLC) and metastatic HCC (MHCC97L and MHCC97H) cells were generated by injecting tumor cells into nude mice s.c. At 3 to 4 weeks after injection, tumor tissue was fixed in formalin buffer at 4°C overnight and embedded in paraffin blocks.

**Statistical analysis.** Statistical analysis was done using SPSS version 14.0. The association between Cdc25A expression and clinical variables of the patients was assessed using the χ2 test, and P < 0.05 was regarded as statistically significant.

**Results**

Metastatic HCC cells showed defective S-M phase checkpoints in response to cisplatin treatment. To determine whether...
metastatic cancer cells show any different cell cycle checkpoint features from nonmetastatic cells, we compared S and G/M checkpoints between Hep3B (nonmetastatic) and MHCC97L (metastatic) HCC cells in response to cisplatin-mediated DNA damage. Throughout the observation period, a baseline of 35% to 40% nonmetastatic Hep3B cells was in S phase as detected by bromodeoxyuridine labeling. After cisplatin treatment, the population in S phase increased to 78% at 24 h and 65% at 48 h, indicating a strong S phase delay after cisplatin treatment (Fig. 1A). In contrast, metastatic MHCC97L (97L) cells did not show an obvious change in the S phase population in response to cisplatin treatment, with 28% S phase without cisplatin to 38% after cisplatin at 24 h and 31% to 39% at 48 h, respectively (Fig. 1B).

We next examined the G2-M checkpoint in the two cell lines in response to cisplatin-mediated damage. As most hepatocytes easily lose diploid features, we tested the G2 checkpoint by counting the phospho-histone H3 (Ser10)-positive mitotic cells. A comparison of the mitotic index of the nonmetastatic and metastatic HCC cell lines following cisplatin treatment is shown in Fig. 1C. The mitotic fraction of Hep3B cells was markedly reduced from 3.63% at 0 h to 0.59% at 36 h after cisplatin treatment, whereas the mitotic fraction of 97L cells remained a significantly higher level up to 36 h (Fig. 1C and D). Consistent with these results, the protein level of phospho-histone H3 disappeared after cisplatin treatment in Hep3B cells, whereas it remained unchanged before and after drug addition in 97L cells (Fig. 1E). The results indicated that metastatic 97L cells did not have an effective G2 checkpoint and they continued to enter mitosis.

**Cisplatin treatment failed to trigger Cdc25A degradation in metastatic HCC cells.** Cdc25A is a critical regulator of the cell cycle machinery. Because Cdc25A can activate cyclin E (A)/CDK2 and cyclin B1/CDK1 complexes by dephosphorylating CDKs, we hypothesized that drug-induced changes in Cdc25A might be linked to S-M phase arrest in these cell lines. In Hep3B cells, treatment with 1 μg/mL cisplatin resulted in a pronounced reduction in the level of Cdc25A protein 12 to 24 h after drug addition (Fig. 2A). The Cdc25A degradation pattern and kinetics were similar to other cancer cell lines treated with cisplatin (7). In contrast, in two metastatic cell lines 97L and 97H cells, Cdc25A levels remained unchanged after the same amount of cisplatin treatment (Fig. 2A). Increasing the dosage of cisplatin from 2 to 6 μg/mL at 12 h of treatment failed to induce a detectable change of Cdc25A (Fig. 2B). We confirmed these results in another paired nonmetastatic (H2P) and metastatic (H2M) HCC cell lines (17), in which cisplatin induced dramatic Cdc25A reduction in H2P but not in H2M (Fig. 2C). Shown by two paired nonmetastatic and metastatic HCC cell lines, deregulation of Cdc25A activity seemed to be unique to metastatic HCC cells. Thus, defective S phase delay and premature mitotic entry in metastatic HCC cells after cisplatin treatment could result from deregulation of Cdc25A degradation.

**Activation of Chk1 and Chk2 was similar in nonmetastatic and metastatic HCC cells.** Cdc25A is targeted for degradation by Chk1 and Chk2 kinases (5, 23). Alkylating agents have been found to activate the ATM/ATR-Chk1/Chk2-Cdc25A pathway (7), so we used cisplatin treatment to investigate Chk1 and Chk2 phosphorylation in nonmetastatic and metastatic HCC cell lines. Chk1 phosphorylation on Ser345 and Ser317 was observed in both lines (Fig. 3A). Chk2 was phosphorylated on Thr68 in both cell lines (Fig. 3B). Thus, both Hep3B and 97L showed similar patterns of cisplatin-induced Chk1 and Chk2 phosphorylation, with the timing accounting for the onset of Cdc25A degradation for Hep3B cells (Figs. 2A and 3).

**Plk1 was not down-regulated after DNA damage in metastatic HCC cells.** Because phosphorylation of upstream activators Chk1 and Chk2 was normal in both cell lines, we questioned whether other DNA damage response proteins could act as...
upstream activators to contribute to the deregulation of Cdc25A degradation in metastatic HCC cells. Plk1 and Plk3 of the Plk family have been found to function at DNA damage checkpoints by regulating Cdc25 (11-14). We therefore sought to determine the potential role of Plk1 and Plk3 in our experimental setup. Nonmetastatic (Hep3B and PLC) and metastatic (97L and 97H) HCC cells were exposed to 1 μg/mL cisplatin for 24 h; levels of Plk1 and Plk3 mRNA and protein were examined. As shown in Fig. 4A and B, cisplatin treatment dramatically reduced the expression of Plk1 at the mRNA and protein levels in Hep3B cells and also Plk1 protein level in PLC cells, consistent with previous observations (24). However, both mRNA and protein levels of Plk1 showed no change when this procedure was carried out with 97L and 97H cells. These results indicated that cisplatin-mediated DNA damage did not trigger down-regulation of Plk1 in metastatic cells. Plk3 responses to cisplatin were also examined. Although the mRNA level of Plk3 seemed to be slightly enhanced by cisplatin treatment in Hep3B cells, the protein levels were basically the same before and after treatment in all cell lines tested (Fig. 4A and B).

Abnormal Plk1-Cdc25A pathway in metastatic HCC cells. As Plk1 was not down-regulated in response to cisplatin-mediated DNA damage in metastatic HCC cells, we examined whether there was any correlation between deregulation of Plk1 and deregulation of Cdc25A degradation. To do so, we investigated Cdc25A degradation in 97L HCC cells in response to cisplatin when Plk1 was knocked down using small interfering RNA. As shown in Fig. 5A, in the background of small interfering control transfected cells, Cdc25A degradation did not occur even after 12 h of exposure to cisplatin. When Plk1 was almost completely depleted by small interfering Plk1 transfection, the level of Cdc25A was markedly reduced after 12 h of cisplatin treatment, indicating a correlation between the expression levels of Plk1 and Cdc25A. Furthermore, after only 6 h of cisplatin treatment, whereas Hep3B cells (Fig. 2A) and other cancer cells (7) did not show an obvious decrease in Cdc25A, 97L HCC cells with knocked down Plk1 showed a dramatically reduced level of Cdc25A (Fig. 5A), suggesting that Plk1 might directly regulate Cdc25A. It was noticed that Cdc25A degradation was dramatic even when there was a residual level of Plk1 after small interfering Plk1 transfection (Fig. 5A, lane 5), which might be due to synergistic effect by both Plk1 down-regulation and cisplatin treatment. We further investigated the correlation in baseline expression between Plk1 and Cdc25A. The expression levels of Plk1 and Cdc25A in four HCC cell lines appeared to be interrelated in the condition without DNA damage (Fig. 5B). This correlated expression pattern was further proven in primary HCC tumor samples, with deregulated high expression of Cdc25A strongly associated with high level of Plk1 (Fig. 5C). Thus, we showed that Plk1 is an upstream regulator of Cdc25A.

![Fig. 2. Effect of cisplatin on Cdc25A degradation.](image1)

![Fig. 3. Activating phosphorylation of Chk1 and Chk2 after cisplatin treatment.](image2)
(Fig. 5D), and its deregulation after DNA damage might lead to deregulation of Cdc25A degradation.

**Biological effects of Cdc25A deregulation.** Based on these results, metastatic HCC cells showed deregulation of Cdc25A degradation and a defective S-M phase checkpoint in response to cisplatin treatment, which may have biological consequences for the cells. Absence of S phase delay and premature mitotic entry following DNA damage usually result in more cell death. Interestingly, metastatic 97L and 97H cells did not show increased sensitivity; rather, they were more resistant to cisplatin when compared with nonmetastatic Hep3B and PLC cells in a cellular survival assay (Fig. 6A). This unexpected result suggested that metastatic HCC cells lack efficient cell cycle delay in response to DNA damage, so they continued to divide and produce cells containing unrepaired DNA damage. To test this possibility, we examined the levels of γ-H2AX, a marker for...
Fig. 6. Biological effects of Cdc25A deregulation. 
A, cisplatin sensitivity by MTT assay. Survival fraction (%) was counted as the activity of dehydrogenases at 450 nm absorbance with cisplatin treatment versus without cisplatin treatment. Mean ± SD of two experiments. 
B, H2P, H2M, Hep3B, PLC, 97L, and 97H cells were stained with anti-γ-H2AX antibody. γ-H2AX foci positive cells (%) were mean ± SD from two repeated staining with each counting 500 to 600 cells. Photos were represented γ-H2AX foci images. 
C, tumor sections derived from nonmetastatic (PLC) and metastatic (97L) HCC cells were stained with anti-γ-H2AX antibody, and positive signals (brown) were visualized using polymer horseradish peroxidase-conjugated antibody. Light blue, nuclear by counterstaining. Percentage of γ-H2AX-positive cells was determined by counting five random fields. 
D, γ-H2AX (top) and Cdc25A (bottom) staining of HCC patient tumors (1167T and 1260T). Left, -; right, ++. 

A, cisplatin sensitivity by MTT assay. Survival fraction (%) was counted as the activity of dehydrogenases at 450 nm absorbance with cisplatin treatment versus without cisplatin treatment. Mean ± SD of two experiments. 
B, H2P, H2M, Hep3B, PLC, 97L, and 97H cells were stained with anti-γ-H2AX antibody. γ-H2AX foci positive cells (%) were mean ± SD from two repeated staining with each counting 500 to 600 cells. Photos were represented γ-H2AX foci images. 
C, tumor sections derived from nonmetastatic (PLC) and metastatic (97L) HCC cells were stained with anti-γ-H2AX antibody, and positive signals (brown) were visualized using polymer horseradish peroxidase-conjugated antibody. Light blue, nuclear by counterstaining. Percentage of γ-H2AX-positive cells was determined by counting five random fields. 
D, γ-H2AX (top) and Cdc25A (bottom) staining of HCC patient tumors (1167T and 1260T). Left, -; right, ++.
damaged DNA foci, in nonmetastatic (H2P, Hep3B, and PLC) and metastatic (H2M, 97L, and 97H) HCC cell lines under a condition without any DNA damage reagent treatment. As shown in Fig. 6B, the percentages of γ-H2AX foci-positive cells in metastatic HCC cell lines were significantly higher than those in nonmetastatic HCC cell lines, which was in accordance with the results of tumor sections generated from 97L cells (20.5%) compared with sections from PLC cells (1.4%; Fig. 6C). The results were in consistence and indicated that metastatic HCC cells had more accumulated damaged DNA than nonmetastatic HCC cells, which might be due to checkpoint adaptation. Moreover, we determined γ-H2AX foci in primary HCC tumors with or without venous infiltration. As shown in Table 1, 5 of 6 metastatic HCC tumor sections exhibited positive γ-H2AX foci, whereas only 1 of 5 nonmetastatic HCC sections showed γ-H2AX foci, indicating that the checkpoint adaptation indeed occurred in vivo and preferentially in metastatic HCC patients (Fig. 6D).

**Aberrant Cdc25A expression was associated with HCC metastasis in clinicopathologic study.** Because we observed the deregulation of Cdc25A and degradation in metastatic HCC cells, we next investigated whether there was correlation between Cdc25A expression and tumor metastasis in 30 HCC patients. Three variables, tumor-node-metastasis staging, venous infiltration, and large tumor size (>5 cm), were significantly associated with expression of Cdc25A, with higher levels of Cdc25A (++ to ++++) found in HCC patients with tumor-node-metastasis stage III to IV and positive venous infiltration (Table 2). Thus, aberrant accumulation of Cdc25A was not only found in metastatic HCC cell lines but was also strongly associated with tumor invasiveness and metastasis in HCC patients. Cdc25A staining and representative signal intensity on HCC tumor section were shown in Fig. 6D. It was noted that Cdc25A levels was also associated with levels of γ-H2AX (Table 1; Fig. 6D).

**Discussion**

Currently, studies regarding tumor metastasis mechanisms focus mainly on molecular regulation of genes involved in cell motility in vitro and in vivo. However, the biological characteristics of metastatic tumor cells, such as the cell cycle regulation, which is critical to cell fate, were rarely examined. For the first time, the present study characterized the cell cycle checkpoints and their regulation in metastatic HCC cells. We identified that, when subjected to cisplatin treatment, metastatic HCC cells displayed a defective S-M phase checkpoint so that DNA synthesis was not slowed down and the cells continued to enter into mitosis. Cisplatin-mediated degradation of Cdc25A was abnormal in metastatic HCC cells and correlated with the observed defective S-M phase delay. The cisplatin treatment in these metastatic cells activated Chk1 and Chk2 by phosphorylation as normal, but it failed to induce Plk1 inhibition. Using the RNA interference approach, we showed that Plk1 could be an upstream regulator of Cdc25A; therefore, a deregulated Plk1-Cdc25A pathway may contribute to defective cell cycle checkpoints and to cell cycle adaptation in metastatic HCC cells.

An intriguing observation of the present study is that although metastatic HCC cells were defective in a cisplatin-mediated cell cycle checkpoint, the lack of a cell cycle delay did not enhance DNA damage-mediated cell death. The cells passed over both DNA damage-induced cell cycle delay and the mitotic catastrophe. They continued to divide with unrepaired DNA damage (checkpoint adaptation) as evidenced by a strikingly high proportion of γ-H2AX-positive DNA damage foci in metastatic HCC cells as well as in vivo in primary metastatic HCC tumor (Fig. 6B-D; Table 1). Checkpoint adaptation can facilitate the elimination of cells with excessively damaged DNA by cell death (16), but some cells survive with accumulated DNA lesions. The latter seems to be the case with metastatic HCC cells. Defects in DNA damage checkpoints are not uncommon in cancer cells, but their occurrence in combination with checkpoint adaptation might contribute to antiapoptosis and chemoresistance, allowing metastatic cancer cells to survive to end stage. Therefore, it would be of great interest to further investigate checkpoint adaptation and its regulation in metastatic cancer cells.

Overexpression of Cdc25A is common in many kinds of cancers (8, 9). Our study is the first to show that aberrant Cdc25A expression is strongly associated with metastatic HCC disease. Most HCC patients who we examined with aberrantly high expression of Cdc25A were at tumor-node-metastasis stage

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<th>Clinical variable</th>
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*P < 0.05 was considered statistically significant.
III to IV, with tumor invasion to a portal or hepatic vein, and adjacent or even distant organs (Table 1). More importantly, we further showed distinct proliferating features in metastatic HCC cells such as the lack of cisplatin-mediated $S$ and $G_2$ checkpoints and the accumulation of DNA damage foci resulting directly or indirectly from an abnormally high level of Cdc25A. Overexpression of Cdc25A, which promotes cell cycle progression, could be correlated with the rapid proliferation rate of cancer cells (9). Our results suggested that, in metastatic HCC cells, it might be correlated more with the accumulation of abnormal chromosomes due to the lack of proper checkpoint control.

The ATM/ATR-Chk1/Chk2-Cdc25A pathway is a classic DNA damage response cascade leading to Cdc25A degradation and consequently cell cycle delay (1). This is also proven to be the case for Hep3B cells after cisplatin treatment (Figs. 2A and 3). The metastatic cell line MHCC97L showed a similar pattern and kinetics of Chk1 and Chk2 phosphorylation as Hep3B cells in response to cisplatin treatment, but the level of Cdc25A never diminished and might even have been slightly enhanced (Figs. 2A and 3), therefore, factors other than Chk1 and Chk2 may be more critical in regulating Cdc25A degradation in metastatic HCC cells. In an effort to search for other potential upstream regulators of Cdc25A, we showed an association between DNA damage-mediated Plk1 inhibition and Cdc25A down-regulation. This is based on several lines of evidence: (a) in response to cisplatin treatment, metastatic HCC cells showed aberrant accumulation of both Plk1 and Cdc25A (Figs. 2A-C and 4A and B); (b) when Plk1 was down-regulated by RNA interference, Cdc25A was rapidly degraded in response to cisplatin treatment, suggesting that Cdc25A could be a direct target of Plk1 (Fig. 5A); and (c) high expression levels of Plk1 and Cdc25A appeared to be correlated in both HCC cell lines (Fig. 5B) and primary HCC samples (Fig. 5C). Plk1 is a multifunctional kinase regulating cell division (25, 26). As a DNA damage checkpoint protein, it is catalytically inactivated (12) to inhibit the cyclin B-CDK1 complex probably through Cdc25C nuclear translocation (25). By the small interfering RNA approach, we showed for the first time that Cdc25A was also a target of Plk1 in that inhibition of Plk1 effectively triggered the DNA damage-mediated down-regulation of Cdc25A (Fig. 5A). A potential Plk1-Cdc25A pathway is proposed in Fig. 5D: DNA damage induces ATM/ATR-dependent inhibition of Plk1, which down-regulates Cdc25A and leads to further activation of checkpoint effectors.

Plk1 has been shown to be an essential component of checkpoint adaptation in mammalian cells (15, 16). If accumulated DNA damage lesions observed in metastatic HCC cells are the result of checkpoint adaptation, it would be of interest to elucidate whether Plk1, which is highly expressed in metastatic HCC cells, plays a role in “aberrant adaptation.” Recently, Plk1 has received increased attention as a putative cancer treatment target after a small-molecule inhibitor of Plk1 showed significant antitumor activity in vivo (27, 28). The deregulated Plk1-Cdc25A pathway in metastatic HCC cells might provide the molecular basis for using Plk1 inhibitors as therapeutics to treat metastatic HCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Clinical Cancer Research

Aberrant Polo-Like Kinase 1-Cdc25A Pathway in Metastatic Hepatocellular Carcinoma

Xiao Qi Wang, Yong Qiang Zhu, Karen S. Lui, et al.


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