Cisplatin-induced Post-translational Modification of Histones H3 and H4*

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The anti-cancer drug cisplatin kills cells by damaging DNA and inducing apoptosis. Understanding the detailed mechanisms by which cancer cells respond to cisplatin has the potential to improve substantially platinum-based therapy. Post-translational modification of histones alters chromatin structure, facilitating the binding of nuclear factors that mediate DNA repair, transcription, and other processes. In the present study, we have investigated the effects of cisplatin treatment on histone post-translational modification in cancer cells. We discovered that specific phosphorylation of histone H3 at Ser-10, mediated by the p38 MAPK pathway, is induced in response to cisplatin treatment. In addition, hyperacetylation of histone H4 was caused by drug treatment. These findings revealed a link between cisplatin administration and chromosomal structural alterations, providing mechanistic information about how cells respond to platinum-induced stress.

cis-Diaminedichloroplatinum(II) (cisplatin or cis-DDP),1 widely used to treat of a variety of human cancers, binds to DNA to form covalent platinum-DNA adducts (1, 2). Platinization of the genome triggers cellular responses involving several pathways, including DNA repair, transcription inhibition, cell cycle arrest, and apoptosis, all of which require remodeling of the structural and dynamic properties of chromatin (3–6).

The p38 mitogen-activated protein kinase (MAPK) pathway has been implicated in osmotic stress, cell cycle regulation, differentiation, inflammation, development, and many other biological processes (7–12). In particular, the p38 MAPK pathway is involved in the cellular response to various DNA-damaging agents, including UV irradiation and cisplatin (13–15). Recently, a growing body of evidence suggests that activation of the p38 MAPK pathway plays an important role in the therapeutic response to cisplatin (16, 17). Cisplatin, but not the inactive stereoisomer trans-DDP and not the Pt(IV) compound PtCl4, induces long term activation of p38 MAPK (16). This kinase regulates immediate early gene expression and other cellular responses by phosphorylating various substrates, including transcription factors, chromatin proteins, and downstream Ser/Thr effector kinases (18). Understanding the detailed mechanism of signal transduction pathways that arise when cells respond to cisplatin treatment could lead to the rational design of better drugs and more efficient therapy.

Histone post-translational modification regulates chromatin structure and cell division (19–23). Phosphorylation of histone H3 at Ser-10 has dual and opposing roles in interphase and metaphase. During interphase, phosphorylation of H3 at Ser-10 can facilitate transcription of the immediate early genes (24–28), whereas during mitosis, such phosphorylation facilitates chromosome remodeling and condensation (29–31). Several stimuli including UV irradiation, anticancer treatment, and epidermal growth factor (EGF) bring about phosphorylation of histone H3, but until now, it was unknown whether cisplatin could induce such phosphorylation. In the present study, we investigated the phosphorylation state of histone H3 in cells treated with cisplatin as well as the signal transduction pathway mediating such an event. We discovered that the drug triggers specific phosphorylation of histone H3, mediated by the p38 MAPK pathway. We further report that hyperacetylation of histone H4 also occurred. These findings reveal a direct link between cisplatin treatment and chromosomal modifications that can lead to structural changes with attendant modulation of function.

MATERIALS AND METHODS

Reagents—Cisplatin was obtained as a gift from Johnson-Matthey. Antibodies were purchased from Upstate Biotechnology. Sodium butyrate was purchased from Sigma. Dulbecco’s modified Eagle’s medium, RPMI 1640 medium, and L-glutamine were obtained from Invitrogen. SKF86022 was bought from Calbiochem.

Cell Culture—HeLa and MCF-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum on tissue culture plates at 37 °C in a humidified atmosphere of 5% CO2. Ntera II cells were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum and 2 mM glutamine on tissue culture plates at 37 °C in a humidified atmosphere of 5% CO2. The cells were grown in medium to near 90% confluence and then pretreated with various concentrations of cisplatin for fixed times before the preparation of nuclear extracts. For studies with the p38 MAPK inhibitor SKF86022, cells were pre-incubated with 10 μM of the inhibitor for 1 h before cisplatin treatment.

Preparation of Nuclear Extracts—Nuclear extracts were prepared from cells as described in the Clontech TransFactor extraction kit (P0812-2) with a slight modification. Briefly, HeLa cells were washed twice with 5 packed cell volumes of cold phosphate-buffered saline. The cells were collected and transferred to clean centrifuge tubes and then centrifuged for 5 min at 450 × g, after which the pellets were rinsed twice with an equal volume cold phosphate-buffered saline. The cell pellets were resuspended with 5 packed cell volumes of ice-cold lysis buffer (10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 1 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride) and incubated on ice for 15 min. The cells were spun down, and the pellets were resuspended with 2 packed cell volumes of ice-cold lysis buffer. The cells were lysed with 10 strokes of a narrow gauge syringe (catalog number 263/4). The suspension was spun at 11,000 × g for 20 min. The pellet was resuspended in a two-thirds volume extraction buffer comprising 20 mM Hepes, pH 7.9, 25% glycerol, 0.42 mM NaCl, 1 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride, and 1.5 mM MgCl2. The solution was

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1 The abbreviations used are: cisplatin or cis-DDP, cis-diaminedichloroplatinum(II); MAPK, mitogen-activated protein kinase; JNK, c-Jun NH2-terminal kinase; ERK, extracellular signal-regulated kinase; EGFR, epidermal growth factor; MAP, mitogen-activated protein.
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RESULTS

Cisplatin Strongly Induces Histone H3 Ser-10 Phosphorylation in HeLa Cells—To study whether cisplatin activates a pathway leading to phosphorylation of histone H3 at Ser-10, HeLa nuclear extracts were analyzed by Western blot using an antibody against phospho-Ser-10 of histone H3 (Fig. 1, lane 1). HeLa nuclear extract (control); lane 2, HeLa nuclear extract pretreated with 1 μM cisplatin overnight. B, time course (t) of cisplatin-induced phosphorylation of histone H3 Ser-10. HeLa nuclear extracts were prepared from cells pretreated with 10 μM cisplatin for 0, 1, 4, 8, 12, or 24 h. C, dose dependence of cisplatin-induced phosphorylation of histone H3 Ser-10. HeLa cells were treated with 0, 0.2, 1.0, 10, or 40 μM cisplatin for 8 h before harvesting.

Proteins were analyzed by SDS-PAGE on a 4–20% Tris-HCl minigel (Bio-Rad). Membranes containing proteins were incubated with specific antibodies as indicated in the figures and with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibody separately and were detected with the ECL plus detection system (Amer sham Biosciences). Actin was used as the loading control.

Cisplatin Induces Histone H3 Ser-10 Phosphorylation in Other Cell Lines—To address whether this cisplatin-induced phosphorylation of histone H3 at Ser-10 is cell-specific, we also tested MCF-7 breast cancer cells and the Ntera II testicular cancer cells. Cisplatin induces phosphorylation of histone H3 at Ser-10 in both of these cell lines. Interestingly, the induction of histone H3 phosphorylation was significantly greater in the Ntera II than the MCF-7 cells.

SKF86002 Inhibits Phosphorylation of H3 at Ser-10 Activated by Cisplatin—Because we discovered that cisplatin treatment strongly induces phosphorylation of histone H3 at Ser-10 in HeLa cells. Phosphorylation of this amino acid is associated with mitotic and meiotic chromosome condensation (22, 29, 31), helps to regulate transcription (24), and is brought about by various stimuli, including 12-O-tetradecanoylphorbol-13-acetate, EGF, UV irradiation, and arsenite (27, 32, 33).

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DISCUSSION

In the present study, we discovered that cisplatin treatment strongly induces phosphorylation of histone H3 at Ser-10 in HeLa cells. Phosphorylation of this amino acid is associated with mitotic and meiotic chromosome condensation (22, 29, 31), helps to regulate transcription (24), and is brought about by various stimuli, including 12-O-tetradecanoylphorbol-13-acetate, EGF, UV irradiation, and arsenite (27, 32, 33). Cisplatin, a potent anti-cancer drug, forms platinum-DNA adducts and causes a variety of cellular responses. However, a relationship between cisplatin treatment and histone H3 modification had not previously been established.

Cisplatin activates p38 MAPK for 8–12 h in sensitive cells and transiently for 1–3 h in resistant cells (16, 17). Moreover, lack of p38 MAPK activation/function induces a resistant phenotype in human cells (16, 17). MAP kinases may also play a role in modifying the chromatin environment of target genes. Here we show that SKF86002, a potent p38 inhibitor, inhibits cisplatin-induced phosphorylation of histone H3 at Ser-10. Furthermore, the p38 MAP kinase and downstream kinase MSK-1 have been reported to phosphorylate histone H3 in vitro (13, 27). Taken together, this evidence indicates that p38 MAPK mediates phosphorylation of histone H3 at Ser-10 in response to cisplatin.

Our results also indicate that the inactive isomer of cisplatin, trans-DDP, fails to induce phosphorylation of histone H3 at Ser-10. Previous work revealed that both cisplatin and trans-DDP activate p38 MAPK differently. Only the active drug cisplatin can bring about long term activation (16). This difference in the kinetics of activation could account for their differential cytotoxicity.

Cisplatin also modestly induces phosphorylation of histone...
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Fig. 4. Cisplatin induction of phosphorylation of H3 Ser-28 and acetylation of H4. A, HeLa nuclear extracts were analyzed by Western blot using antibody against phospho-Ser-28 of histone H3. Lane 1, HeLa nuclear extract (control). Lane 2, HeLa nuclear extract pretreated with 1 μM cisplatin overnight. B, nuclear extracts prepared from HeLa cells were analyzed by Western blot using anti-acetyl-lysine antibody or anti-acetylated histone H4 as indicated. Lane 1, HeLa nuclear extract (control). Lane 2, HeLa nuclear extract pretreated with 1 μM cisplatin overnight. Lane 3, HeLa nuclear extract pretreated overnight with 4 mM sodium butyrate, an inhibitor of histone deacetylases (positive control).

H3 at Ser-28. Phosphorylation at this site can also be triggered by UV irradiation, Trichostatin A, and other stimuli (34, 35). The kinetics are very similar to those for Ser-10. Both Ser-10 and Ser-28 on histone H3 N-terminal tails are highly conserved and occur in the same consensus sequence, ARKS (22).

There are two distinct modes of histone H3 phosphorylation, mitotic and stimulus-inducible. Both Ser-10 and Ser-28 on histone H3 are highly phosphorylated on condensed chromosomes during mitosis. The exact role of histone phosphorylation is still not fully understood, although several models have been proposed to explain such a process during mitosis (30). Diverse stimuli induce rapid phosphorylation of H3 at Ser-10, which has been correlated with transcription activation of certain immediate early genes, by changing the nucleosome environment or serving as a binding motif for recruiting co-activators or chromatin remodeling complexes.

Cisplatin-induced phosphorylation of histone H3 also occurs in cell types other than HeLa. In testicular cancer cell lines, a high level of H3 phosphorylation is observed. Because the Ntera II cell line is at least twice as sensitive to cisplatin as MCF-7 cells (data not shown), there may be a link between H3 phosphorylation and cisplatin cytotoxicity.

The acetylation of histones correlates well with the transcription activation of many genes (36), presumably by increasing the accessibility of particular genomic regions for transcription activator or remodeling protein complexes. The acetylation of histones is under the control of histone acetyltransferases and histone deacetylases. We find that cisplatin treatment induces hyperacetylation of histone H4. Although this process could occur by a mechanism independent from that by which histone H3 is phosphorylated, it might be that MAP kinase-dependent recruitment of co-activators with histone acetyltransferase activity to sequence-specific regulatory elements contributes to the acetylation of histones, as has been reported previously (37, 38). Acetylation and phosphorylation of histones are linked with transcriptional activation and facilitate DNA repair (39, 40).

Phosphorylation of histone H3 Ser-10 is also induced by UV irradiation, arsenite, EGF, and other stimuli through activation of the MAPK pathway (Table I) (18). In general, the extracellular signal-regulated kinases (ERKs) are activated by mitogenic and proliferative stimuli, such as EGF and 12-O-tetradecanoylphorbol-13-acetate. The c-Jun NH2-terminal kinases (JNKs) and p38 MAPKs respond to environmental stress and chemotherapeutic drugs, such as UV irradiation, arsenite, and cisplatin. The different patterns of MAPK pathway activation caused by these various stimuli may represent a regulatory signal by which cells respond to stress in a stimuli-specific manner.

Besides p38 MAPK, both JNK and ERK1/2 have been implicated in the cellular response to cisplatin (41–43). ERK was weakly activated (~2–3-fold) by a 33 μM cisplatin treatment for 24 h, whereas JNK was more significantly activated (10-fold) (41). Another study showed no activation of ERK1/2 in HaCaT cells following a 33–132 μM cisplatin treatment for 4 h, whereas JNK and p38 MAPK could be activated by 33 μM cis-DDP (16). In addition, a third group reported no activation of ERK1/2 in HeLa cell lines after a 10 μM cisplatin treatment for 12 h, even though ERK activation was observed at higher cisplatin concentrations (43). Taken together, these results make it unlikely that ERK1/2 was activated by cisplatin under the same experimental conditions (1 μM) in which H3 was phosphorylated. Although we do not exclude other kinases for the cisplatin-induced phosphorylation of histone H3, the MAPK pathway seems to be a prominent one.

Cisplatin-DNA adducts arrest the cell cycle (44–46). Nucleotide excision repair complexes are then recruited to repair the DNA damage. p38 MAPK-mediated phosphorylation and acetylation of histones cause nucleosomal structural changes, increasing accessibility of other proteins. These events facilitate the binding of the remodeling complex and repair proteins to the nucleosome, thereby stimulating DNA repair (39, 40, 47) and helping cells to survive cisplatin-induced stress. When DNA damage is extensive, cells will undergo apoptosis. Phosphorylation and acetylation of histones leading to the activation and transcription of immediate early genes will facilitate apoptosis under these conditions.

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**TABLE I**

**Stimuli that activate a MAPK pathway to induce histone phosphorylation**

| Stimulus | Activation of MAPK pathway | Histone modification |
|----------|-----------------------------|----------------------|
| Cisplatin | p38                         | H3 Ser-10/Ser-28 phosphorylation |
| UV       | p38, ERK                    | Unknown              |
| Arsenite | p38, ERK2                   | H3 Ser-10 phosphorylation |
| EGF      | ERK                         | H3 Ser-10 phosphorylation |
| TPA      | ERK                         | H3 Ser-10/Ser-28 phosphorylation |

* TPA, 12-O-tetradecanoylphorbol-13-acetate.
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