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The enhancement effect of *HIST1H4C* knockdown on cadmium toxicity in human proximal tubular cells

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ABSTRACT — Cadmium (Cd) is a toxic heavy metal known to exert severe nephrotoxic effects. Mechanistically, Cd has been reported to disrupt gene expression in renal proximal tubular cells. In addition, alterations in DNA integrity have been reported to be associated with Cd toxicity. Histone proteins play important roles in maintaining DNA integrity, and are responsible for regulating gene transcription. In this study, we examined the involvement of *HIST1H4C*, a gene encoding the histone H4 protein, in Cd toxicity in HK-2 human proximal tubular cells. It was found that Cd significantly reduced the transcription level of *HIST1H4C* in HK-2 cells. In addition, *HIST1H4C* knockdown by siRNA transfection enhanced Cd toxicity in HK-2 cells. Our findings suggest that suppression of gene expression of *HIST1H4C* may be involved in the elevation of Cd toxicity in proximal tubular cells.

Key words: Cadmium, HK-2 cells, *HIST1H4C*

INTRODUCTION

Cadmium (Cd) is a toxic heavy metal that exists naturally and is one of the serious pollutants emerging from industrial and agricultural sources (Järup and Akesson, 2009). Cd causes serious dysfunction to various tissues, including the kidney (Järup and Akesson, 2009; Fujiwara *et al.*, 2012). However, the molecular mechanisms underlying Cd-induced renal toxicity remain obscure.

Our previous studies demonstrated that Cd changes gene expressions in human and rat proximal tubular cells, as well as the mouse kidney (Tokumoto *et al.*, 2011, 2013; Lee *et al.*, 2013). Furthermore, Cd adversely affects DNA integrity in various cell lines (Lawal and Ellis, 2010; Liu *et al.*, 2013). These findings indicate that Cd toxicity is associated with DNA alterations.

DNA is enclosed in chromatin, and the chromatin environment plays an important role in the maintenance of DNA integrity (Jackson and Bartek, 2009). Post-translational modification of histones, which form the chromatin core, is essential for the regulation of DNA integrity (Kouzarides, 2007; Lukas *et al.*, 2011). Four core histones (H2A, H2B, H3, and H4) form an octamer around which approximately 146 base pairs (bp) of DNA wraps in repeating units (Ebralidse *et al.*, 1988). Among the histone proteins, histone H4 has been revealed to be critically important for genome integrity, such as DNA damage repair, DNA replication and chromatin compaction (Megee *et al.*, 1995; Jørgensen *et al.*, 2013). In this study, we examined the involvement of *HIST1H4C*, one of genes encoding histone H4 protein, in Cd toxicity in HK-2 human proximal tubular cells.

MATERIALS AND METHODS

Cell culture

HK-2 cells were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 25 U/mL penicillin, 25 µg/mL streptomycin, 1% Insulin-Transferrin-Selenium-X (Gibco), 10 ng/mL EGF (Sigma-Aldrich), and 5 ng/mL hydrocortisone (Sigma-Aldrich) at 37°C in a humidified incubator containing 5% CO_2.

Real-time RT-PCR

HK-2 cells were transferred into 60-mm tissue culture dishes (Falcon, Franklin Lakes, NJ, USA) at a density of 2.0×10^4 cells/cm^2 and were cultured until confluent. Then, culture medium was discarded and the cells were treated with various concentrations of cadmium chloride ($CdCl_2$; Wako Pure Chemical Industries, Osaka, Japan) in

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serum-free medium for 6 hr. Cd-treated HK-2 cells were washed twice with ice-cold phosphate-buffered saline (PBS; Nissui, Tokyo, Japan). Total RNA was extracted using the Quick Gene RNA cultured cell kit S (Fujifilm, Tokyo, Japan). Total RNA was incubated with PrimeScript reverse transcription (RT) Reagent Kit (Perfect Real Time, Takara Bio, Shiga, Japan) to generate cDNA. Real-time PCR was performed with SYBR Premix Ex Taq II (Perfect Real Time, Takara Bio) using the Thermal Cycler Dice Real Time System (Takara Bio). PCR conditions were as follows: 10 sec hot-start at 95°C, followed by 40 cycles of 5 sec at 95°C, and 30 sec at 60°C. Gene expression was normalized to *GAPDH* mRNA levels. The primer oligonucleotide sequences were as follows: sense, 5′-GCACCGTCAAGGCTGAGAAC-3′, and antisense, 5′-TGGTGAAGACGCCAGTGGA-3′, for the human *GAPDH* gene; sense, 5′-CGAGACGCCGTCACCTATAC-3′, and antisense, 5′-CCCTGACGTTTTAGGGCATA-3′, for the human *HIST1H4C* gene.

siRNA transfection

Double-stranded siRNAs were purchased from Qiagen (Germantown, MD, USA). The siRNA product names were as follows: Hs_HIST1H4C_1; Hs_HIST1H4C_2; Hs_HIST1H4C_3; and Hs_HIST1H4C_4, for human *HIST1H4C*. Control siRNA (Silencer® Select Negative Control #1 siRNA) was purchased from Ambion (Grand Island, NY, USA). The siRNA transfections were performed using Lipofectamine RNAiMAX (Invitrogen, Grand Island, NY, USA). After the siRNA mixture was incubated for 15 min with Lipofectamine RNAiMAX and Opti-MEM® I Reduced Serum Medium (Opti-MEM; Gibco), HK-2 cells were transfected with the siRNA mixture for 24 hr.

Cell viability

HK-2 cells were transferred to a 96-well tissue culture plate (Falcon) at a density of 2.0×10^4 cells/cm^2 with the siRNA mixture [1 nM siRNA, 0.2% (v/v) Lipofectamine RNAiMAX, and 10% (v/v) Opti-MEM]. After 48 hr, cells were incubated with $CdCl_2$ in serum-free culture medium for 24 hr. After treatment, serum-free medium containing $CdCl_2$ was replaced with fresh growth medium containing 10% (v/v) Alamar Blue (Invitrogen) and incubated for 4 hr at 37°C. Fluorescence was measured at an excitation wavelength of 540 nm and an emission wavelength of 595 nm using the DTX880 multimode detector (Beckman Coulter Inc., Brea, CA, USA).

Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). When the F-value was significant ($P < 0.05$), Bonferroni's multiple *t*-test was performed for post-hoc comparisons ($P < 0.05$). Student *t*-test was performed in Fig. 2A ($P < 0.05$).

RESULTS AND DISCUSSION

Among the genes encoding for histone H4 protein, we examined the effect of Cd on the gene expression level of *HIST1H4C* in HK-2 cells. Upon treatment of HK-2 cells with various concentrations of Cd for 6 hr, the mRNA levels of *HIST1H4C* in HK-2 cells were dose-dependently decreased, as compared with those in control cells (Fig. 1). Next, the susceptibility of *HIST1H4C* knockdown cells to Cd toxicity was examined. When *HIST1H4C* siRNA was transfected into HK-2 cells, *HIST1H4C* mRNA levels were reduced by about 10% compared with control cells (Fig. 2A). Furthermore, *HIST1H4C* knockdown HK-2 cells were more susceptible to Cd toxicity than control cells (Fig. 2B). However, the viability of *HIST1H4C* knockdown HK-2 cells was the same as that of control cells without Cd treatment. These results suggest that Cd may intensify its toxicity *via* the inhibition of *HIST1H4C* gene expression in HK-2 cells.

Cd has been reported to have adverse effect on DNA

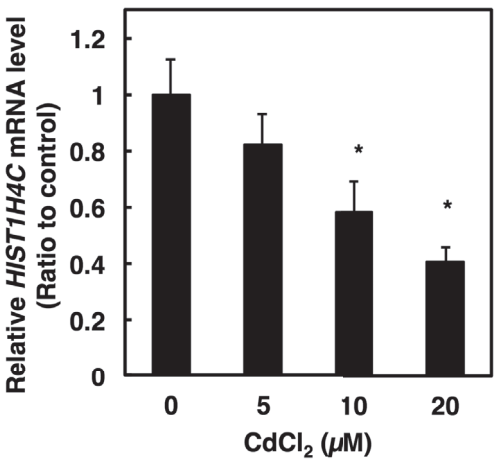

Fig. 1. The effect of Cd on *HIST1H4C* gene expression in HK-2 cells. HK-2 cells were treated with various concentrations of cadmium chloride for 6 hr. The mRNA levels of *HIST1H4C* were measured using real-time RT-PCR. *HIST1H4C* mRNA levels were normalized to *GAPDH* mRNA levels. Values are the means ± S.D. ($n = 3$). *Significantly different from the control, $P < 0.05$.

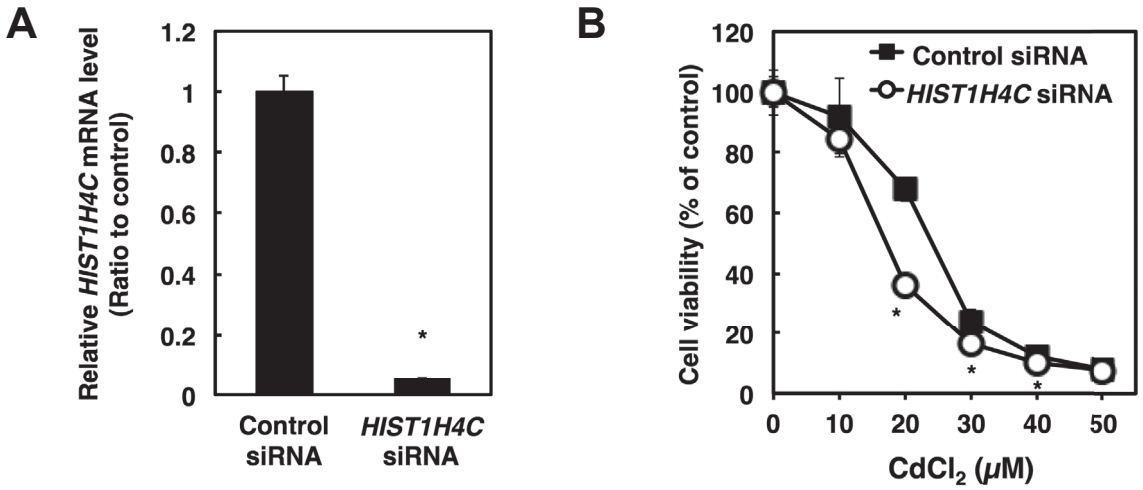

Fig. 2. The effect of *HIST1H4C* knockdown on Cd toxicity in HK-2 cells. (A) The knockdown efficiency of *HIST1H4C* in HK-2 cells following *HIST1H4C* siRNA treatment. *HIST1H4C* siRNA was added to HK-2 cells and then cells were incubated for 48 hr. The mRNA levels of *HIST1H4C* were measured using real-time RT-PCR. *HIST1H4C* mRNA levels were normalized to *GAPDH* mRNA levels. Values are the means ± S.D. (n = 3). (B) The cell viability of HK-2 cells transfected with *HIST1H4C* or control siRNA after treatment with cadmium chloride for 24 hr using the Alamar Blue assay. Values are the means ± S.D. (n = 3). *Significantly different from the corresponding control siRNA group, $P < 0.05$.

integrity (Lawal and Ellis, 2010; Liu *et al.*, 2013). In addition, our previous report demonstrated that Cd disrupts gene expression in kidney cells (Tokumoto *et al.*, 2011, 2013; Fujiwara *et al.*, 2012; Lee *et al.*, 2013). In this study, we found that one of the genes encoding histone proteins, *HIST1H4C*, is involved in the sensitivity of Cd toxicity in HK-2 cells. This is in agreement with previous reports that noted the association between Cd toxicity and the post-translational modification of histone proteins, such as methylation (Gadhia *et al.*, 2015; Xiao *et al.*, 2015). This study suggests that the decrease in the transcription level of histone genes may be involved in Cd toxicity. Further study is needed to identify the precise mechanism between histones and Cd toxicity.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Ebralidse, K.K., Grachev, S.A. and Mirzabekov, A.D. (1988): A highly basic histone H4 domain bound to the sharply bent region of nucleosomal DNA. Nature, **331**, 365-367.

Fujiwara, Y., Lee, J.Y., Tokumoto, M. and Satoh, M. (2012): Cadmium renal toxicity via apoptotic pathways. Biol. Pharm. Bull., **35**, 1892-1897.

Gadhia, S.R., O'Brien, D. and Barile, F.A. (2015): Cadmium affects mitotically inherited histone modification pathways in mouse embryonic stem cells. Toxicol. In Vitro, doi: 10.1016/j.tiv.2015.11.001. (Epub ahead of print).

Jackson, S.P. and Bartek, J. (2009): The DNA-damage response in human biology and disease. Nature, **461**, 1071-1078.

Järup, L. and Akesson, A. (2009): Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol., **238**, 201-208.

Jørgensen, S., Schotta, G. and Sørensen, C.S. (2013): Histone H4 lysine 20 methylation: key player in epigenetic regulation of genomic integrity. Nucleic. Acids Res., **41**, 2797-2806.

Kouzarides, T. (2007): Chromatin modifications and their function. Cell, **128**, 693-705.

Lawal, A.O. and Ellis, E. (2010): Differential sensitivity and responsiveness of three human cell lines HepG2, 1321N1 and HEK 293 to cadmium. J. Toxicol. Sci., **35**, 465-478.

Lee, J.Y., Tokumoto, M., Fujiwara, Y. and Satoh, M. (2013): Gene expression analysis using DNA microarray in HK-2 human renal proximal tubular cells treated with cadmium. J. Toxicol. Sci., **38**, 959-962.

Liu, Q., Gu, J.H., Yuan, Y., Liu, X.Z., Wang, Y.J., Wang, H.D., Liu, Z.P., Wang, Z.Y. and Bian, J.C. (2013): Effect of cadmium on rat Leydig cell testosterone production and DNA integrity *in vitro*. Biomed. Environ. Sci., **26**, 769-773.

Lukas, J., Lukas, C. and Bartek, J. (2011) More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. Nat. Cell Biol., **13**, 1161-1169.

Megee, P.C., Morgan, B.A. and Smith, M.M. (1995): Histone H4 and the maintenance of genome integrity. Genes Dev., **9**, 1716-1727.

Tokumoto, M., Lee, J.Y., Fujiwara, Y. and Satoh, M. (2013): DNA microarray expression analysis of mouse kidney following cadmium exposure for 12 months. J. Toxicol. Sci., **38**, 799-802.

Tokumoto, M., Ohtsu, T., Honda, A., Fujiwara, Y., Nagase, H. and Satoh, M. (2011): DNA microarray analysis of normal rat kidney epithelial cells treated with cadmium. J. Toxicol. Sci., **36**, 127-129.

Xiao, C., Liu, Y., Xie, C., Tu, W., Xia, Y., Costa, M. and Zhou, X. (2015): Cadmium induces histone H3 lysine methylation by inhibiting histone demethylase activity. Toxicol. Sci., **145**, 80-89.