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Toxicomics Report

Effect of cadmium on expression of BIRC family genes in HeLa cells

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ABSTRACT — Cadmium (Cd) is an environmental pollutant with toxic effects in various tissues, including the kidney. In our previous study, Cd changed the expression of baculoviral inhibitor of apoptosis protein repeat-containing (BIRC) family genes in human proximal tubular cells. BIRC family genes inhibit apoptosis, and inducing the apoptosis pathway is an approach for anticancer therapeutics. In this study, the effect of Cd on expression of BIRC family genes was examined in HeLa human cervical cancer cells. Cd treatment affected cell viability, increased *BIRC5* and *BIRC6* mRNA levels, and decreased the *BIRC8* mRNA level in HeLa cells. These results indicate that a low dose of Cd changes the expression of several genes in the BIRC family in HeLa cells. Changes in the mRNA levels of BIRC family genes by exposure to Cd may affect cancer chemotherapeutic agents targeting the apoptosis pathway.

Key words: Cadmium, HeLa cells, BIRC family

INTRODUCTION

Cadmium (Cd) has harmful effects on various tissues, such as the kidney, liver, testis, lung and, bone (Järup and Akesson, 2009; Järup *et al*., 1998; Nordberg *et al*., 2015; Satoh *et al*., 2002). Cd is released into the environment after its industrial use or as a byproduct. Therefore, humans can be exposed to Cd from various food sources and occupationally (Satarug *et al*., 2010). Foods such as rice, seafood, vegetables, animal products, and even chocolate include relatively high concentrations of Cd (Fowler *et al*., 2015). Cd accumulates in the human body over our lifespan, especially in the kidney, because it has a long biological half-life (15–30 years) (Järup and Akesson, 2009; Järup *et al*., 1998; Nordberg *et al*., 2015). Renal proximal tubular cells are the main target of Cd-induced renal toxicity (Järup and Akesson, 2009; Järup *et al*., 1998; Lee *et al*., 2019; Nordberg *et al*., 2015). Our previous study demonstrated that Cd changes the expression of baculoviral inhibitor of apoptosis protein repeat-containing (BIRC) family genes in HK-2 human proximal tubular cells (Lee *et al*., 2017a). Moreover, Cd induces apoptosis by suppression of *BIRC3* expression in HK-2 cells (Lee *et al*., 2017a). Cd also increases *BIRC5* and *Birc5* expression in HK-2 cells and the mouse kidney (Lee *et al*., 2017a, 2017b). *Birc5* expression is slightly induced in the mouse liver exposed to Cd (Lee *et al*., 2017b). BIRC family genes inhibit apoptosis (LaCasse *et al*., 2008). Therefore, changes in the expression of BIRC family genes by exposure to Cd might play an important role in fate determination of cells. Additionally, various anticancer agents target intact apoptotic pathways to trigger cancer cell death (Pistritto *et al*., 2016). Therefore, long-term exposure to low concentrations of Cd may change the

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effects of anticancer agents by affecting the expression of BIRC family genes. In this study using HeLa human cervical cancer cells, we examined the effect of Cd on the expression of BIRC family genes.

MATERIALS AND METHODS

Cell culture and treatment

HeLa human cervical cancer were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Minimum Essential Medium Eagle (EMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 U/mL penicillin (Sigma-Aldrich), and 0.1 mg/mL streptomycin (Sigma-Aldrich) at 37°C in a humidified incubator containing 5% CO_2.

HeLa cells were grown in plates at 500 cells/mm^2 for 24 hr. The culture medium was discarded, and the cells were treated with Cd ($CdCl_2$; Wako Pure Chemical Industries, Osaka, Japan) for 24 hr in serum-free culture medium.

Cell viability assay

HeLa cells were grown in 96-well plates for 24 hr. The culture medium was discarded, and the cells were treated with Cd in serum-free culture medium for 24 hr. After Cd treatment, the culture medium was replaced with 10% FBS-EMEM containing 10% Alamar blue (Invitrogen, Grand Island, NY, USA). After incubation for 4 hr at 37°C, fluorescence was measured at an excitation wavelength of 540 nm and emission wavelength of 595 nm in a SpectraMax® iD3 microplate reader (Molecular Devices, San Jose, CA, USA).

Real-time reverse transcription-polymerase chain reaction (RT-PCR)

HeLa cells were grown in 60-mm dishes for 24 hr. The culture medium was discarded, and the cells were treated with Cd in serum-free culture medium for 15 hr. Cells were washed twice with ice-cold PBS(-) (Gibco), and total RNA was extracted with a PureLink™ RNA Mini Kit (Ambion, Grand Island, NY, USA). The RNA quantity and purity were measured using a BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan). cDNA was generated from the total RNA using a PrimeScript reverse transcription (RT) Reagent Kit (Perfect Real Time) (Takara Bio, Shiga, Japan). Real-time PCR was performed with SYBR Premix Ex TaqII (Perfect Real Time) (Takara Bio) and 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 sequences were as follows: sense, 5′-CATGTGTGTGGAGGGTGAAG-3′ and antisense, 5′-TTTAACAGGGGACAGCATCC-3′′ for the human *BIRC1* gene; sense, 5′-GCATTTTCCCAACTGTCCAT-3′ and antisense, 5′-ATTCGAGCTGCATGTGTCTG-3′ for the human *BIRC2* gene; sense, 5′-CAACAGATCTG-GCAAAAGCA-3′ and antisense, 5′-TTGCTCAATTTTC-CACCACA-3′ for the human *BIRC3* gene; sense, 5′-TGGGGTTCAGTTTCAAGGAC-3′ and antisense, 5′-TGCAACCAGAACCTCAAGTG-3′ for the human *BIRC4* gene; sense, 5′-GTTGCGCTTTCCTTTCTGTC-3′ and antisense, 5′-TCTCCGCAGTTTCCTCAAAT-3′ for the human *BIRC5* gene; sense, 5′-TGACGCTTTCAACCTCACTG-3′ and antisense, 5′-GTGTCCGCTGGACCAGTTAT-3′ for the human *BIRC6* gene; sense, 5′-TGGCCTCCTTCTATGACTGG-3′ and antisense, 5′-ACCTCACCTTGTCCTGATGG-3′ for the human *BIRC7* gene; sense, 5′-AAGCCCGGCTCATTACTTTT-3′ and antisense, 5′-ATCTTCCTTGGGCTTCCAGT-3′ for the human *BIRC8* gene; sense, 5′-GCACCGTCAAGGCTGAGAAC-3′ and antisense, 5′-TGGTGAAGACGCCAGTGGA-3′ for the human *GAPDH* gene.

Statistical analyses

Statistical analyses were carried out using single-factor ANOVA followed by Bonferroni's correction for *post-hoc* comparisons ($P < 0.05$).

RESULTS AND DISCUSSION

To determine the cytotoxicity of Cd, HeLa cells were treated with various concentrations of Cd. Low-dose treatment with Cd for 24 hr did not affect HeLa cell viability (Fig. 1A). However, a high dose of Cd for 24 hr significantly decreased HeLa cell viability (Fig. 1B). Next, the effect of low-dose Cd on expression of BIRC family genes was examined in HeLa cells. Cd treatment did not affect the mRNA levels of *BIRC1*, *BIRC2*, *BIRC3*, *BIRC4*, or *BIRC7* (Figs. 2A–D, G). However, treatment with 0.01 and 0.1 μM Cd significantly increased the *BIRC5* mRNA level (Fig. 2E). Treatment with 0.01 μM Cd also slightly increased the *BIRC6* mRNA level (Fig. 2F). On the other hand, the *BIRC8* mRNA level was decreased by 0.1 and 1 μM Cd treatment (Fig. 2H). These results suggested that non-cytotoxic low doses of Cd changed the expression of several genes in the BIRC family in HeLa cells.

Gene expression of *BIRC5* was increased by Cd treat-

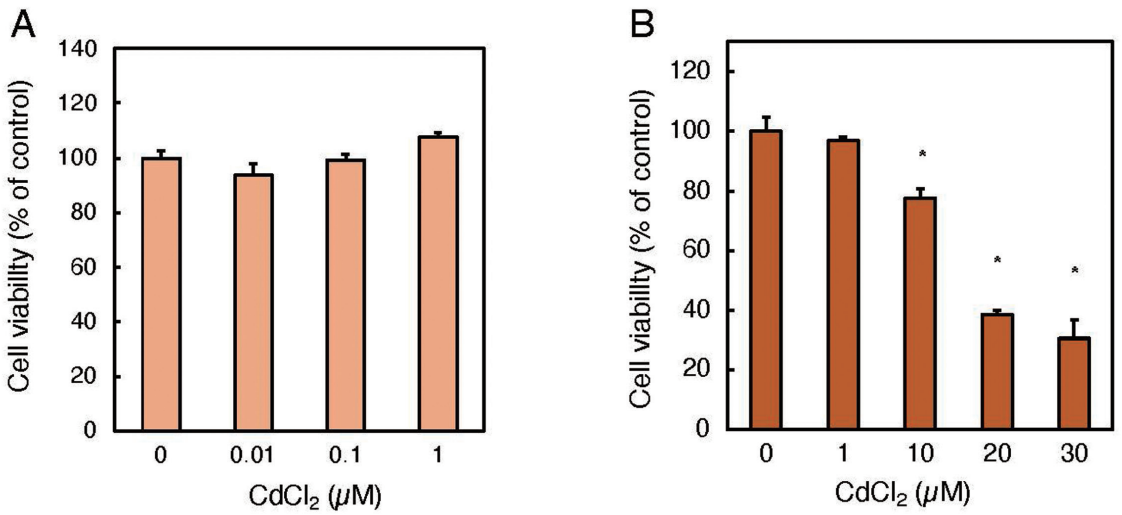

Fig. 1. Effects of Cd on HeLa cell viability. HeLa cells were seeded at 500 cells/mm^2 and cultured for 24 hr. The culture medium was discarded, and cells in serum-free medium were treated with various concentrations of $CdCl_2$ for 24 hr. Cell viability was examined using Alamar blue assays. (**A**) HeLa cells were treated with 0.01, 0.1, and 1 μM Cd. (**B**) HeLa cells were treated with 1, 10, 20, and 30 μM Cd. Values are the mean ± S.D. (n = 5). *$P < 0.05$ compared with the control group.

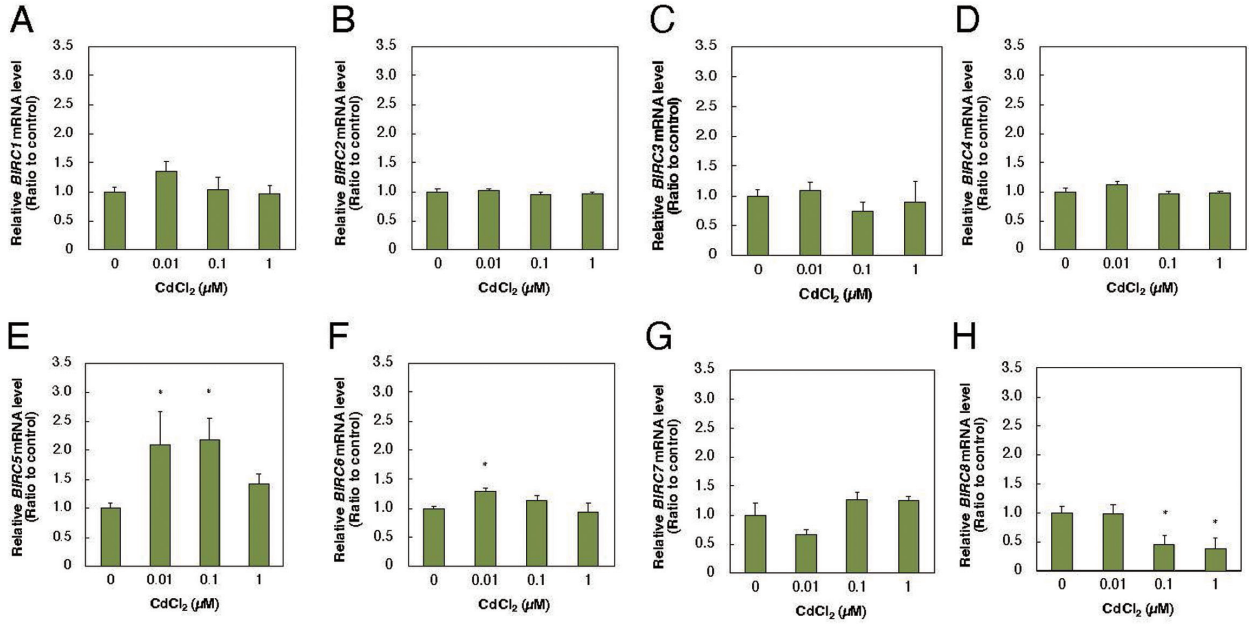

Fig. 2. mRNA levels of BIRC family genes in HeLa cells treated with $CdCl_2$. HeLa cells were seeded at 500 cells/mm^2 and cultured for 24 hr. The culture medium was discarded, and cells in serum-free medium were treated with various concentrations of $CdCl_2$ for 15 hr. mRNA levels were examined by real-time RT-PCR. mRNA levels were normalized to *GAPDH* expression. (**A**) *BIRC1*. (**B**) *BIRC2*. (**C**) *BIRC3*. (**D**) *BIRC4*. (**E**) *BIRC5*. (**F**) *BIRC6*. (**G**) *BIRC7*. (**H**) *BIRC8*. Values are the mean ± S.D. (n = 3). *$P < 0.05$ compared with the control group.

ment in HeLa cells. Our previous study demonstrated that Cd increases *BIRC5* and *Birc5* expression in HK-2 cells and the mouse kidney, respectively (Lee *et al*., 2017a, 2017b). Moreover, methylmercury increases *BIRC5* gene expression in HK-2 cells and IMR-32 human neuroblastoma cells, and inorganic mercury increases *BIRC5* gene expression in HK-2 cells (Lee *et al*., 2018). Therefore, BIRC5 may be a responsive factor against exogenic stress of several metals. The *BIRC5* gene encodes survivin, which is being studied as a cancer therapeutic target (Wheatley and Altieri, 2019). A high level of survivin expression induced by metals in cancer cells may suppress the effect of cancer therapeutics targeting survivin.

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

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