Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.4, No.3, 159-165, 2017

Original Article

Distinct regulation of nuclear localization of caspase-activated DNase during cadmium-induced apoptosis of the target cells

Masami Ishido and Rumiko Usu

Center for Health & Environmental Risk Research National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan

(Received May 1, 2017; Accepted May 9, 2017)

ABSTRACT — We examined whether the nuclear localization of caspase-activated DNase (CAD) are involved in cadmium-induced apoptosis. Exposure of porcine kidney LLC-PK_1 cells to cadmium cleaved poly (ADP-ribose) polymerase (PRAP) as sufficiently as TNF-α did in the renal cells. However, nuclear localization of CAD was not seen during cadmium-induced renal apoptosis, but done during apoptosis by TNF-α. On other hands, in rat splenocytes cadmium could induce nuclear localization of CAD. Thus, our data suggest that activation of caspase is not always to allow the translocation of CAD into the nuclei for the internucleosomal cleavage of DNA, and suggest that the same apoptotic stimulus by cadmium may utilize different sets of caspase cascades or unknown death pathways which are coupled to the caspase proteolysis in different cell types.

Key words: Cadmium, Nuclear location, CAD, Renal cells, Splenocytes

INTRODUCTION

Apoptosis is a programmed form of cell death mediating precisely controlled removal of unnecessary, aged or damaged cells and occurs in virtually all mammalian cells at all time (Kerr *et al*., 1972; Haecker and Vaux, 1994). This phenomenon is initiated not only by physiological stimuli but also by an extensive array of nonphysiological agents, but the mechanisms of both apoptotic inducers do not appear to be same (Sun *et al*., 1999). Apoptotic cells undergo multiple changes including membrane blebbing, nuclear condensation, and fragmentation of genomic DNA into nucleosomal fragments. These morphological and biochemical changes are promoted by the combination of proteases and endonucleases.

At least 14 caspase family members have been identified, and they are classified into three subfamilies, based on their structure and substrates specificity (Nagata, 1997; Nicholson and Thornberry, 1997; Cohen, 1997; Salvesen and Dixit, 1997; Thornberry and Lazebnik, 1998; Wolf and Green, 1999). The activation of caspases are initially synthesized as inactive zymogens composed of the prodomain plus large and small subunits. Generation of the active caspases requires sequence-specific proteolytic cleavage to convert the zymogen to a corresponding active enzyme.

Activated caspases can cleave multiple cytoplasmic and nuclear substrates, a process that appear to play a pivotal role in the execution phase of apoptosis. DNA fragmentation associated with apoptosis is induced by the DNA fragmentation factor (DFF), which is activated by caspase, mainly caspase-3 (Liu *et al*., 1997; Enari *et al*., 1998). DFF is composed of two protein subunits, a 40-kDa caspase-activated nuclease (CAD) and its 45-kDa inhibitor (ICAD) (Sakahira *et al*., 1998; Halenbeck *et al*., 1998; Liu *et al*., 1998; Mukae *et al*., 1998; Sakahira *et al*., 1999). Cleavage of CAD/ICAD by caspase-3 releases CAD from its inhibitor leading to the induction of nuclease activity, nuclear condensation, and DNA fragmentation *in vitro* (Zhang *et al*., 1998).

Cadmium is an environmental pollutant which primarily exerts toxicity on the kidney. The development of cadmium-induced renal lesions is characterized by proteinuria and excessive urinary excretion of other substances such as enzymes, amino acids, and glucose (Friberg *et al*., 1979; Nordberg M and Nordberg GF, 2016). Cadmium cytotoxicity was observed as perturbation of energy producing system, cellular membrane defects, and increased

Correspondence: Masami Ishido (E-mail: ishidou@nies.go.jp)

influx of calcium ions at the end-point of toxicity of the metal.

Apoptogenic nature of cadmium has been discovered (Azzouzi *et al*., 1994; Ishido *et al*., 1995; Xu *et al*., 1996; Yan *et al*., 1997; Ishido *et al*., 1998). But, the molecular transduction pathway of the death signal by cadmium is not fully understood (Thevenod and Lee, 2013). It has been reported that there are caspase-dependent and caspase-independent pathways are involved in cadmium-induced apoptosis (Lee *et al*., 2006; Liu *et al*., 2016). In this study, we investigated whether the translocation of CAD into the nuclei are involved in cadmium-induced apoptotic processing.

MATERIALS AND METHODS

Cell culture

Porcine kidney LLC-PK_1 cells (CRL1392; American Type Culture Collection, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's Medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Inc., Rockville, MD, USA), penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. The cells were subcultured (1:4) 2 to 3 times per week. For primary splenocytes culture, nine-week-old male Jcl: Wistar rats were purchased from Clea Japan (Tokyo, Japan). Animals received humane care according to the National Institute for Environmental Studies guidelines. Animals were maintained in stainless-steel cages and fed with a standard laboratory feed (MF diet, Oriental Yeast Co., Tokyo, Japan) and distilled water *ad libitum* at 22°C on a light-dark cycle (12 hr/12 hr) for 1 week. Rat spleens were removed from four individuals. Splenocytes were sparced by gentle teasing through stainless-steel mesh in RPMI 1640 (Life Technologies, Inc.). The cells were washed with fresh serum-free RPMI 1640 medium (Life Technologies, Inc.) by centrifuge. Cell viability of the splenocytes thus obtained was determined to be 90-95% by trypan blue exclusion method.

DNA fragmentation analysis

DNA fragmentation analysis was carried out as described (Wylle, 1980; Ishido *et al*., 1995). The LLC-PK_1 cells were subcultured at 70% confluency in 10 cm-petri dishes and maintained in serum-free medium for 16 hr. Then, the cells were exposed to cadmium for indicated periods at 37°C. For treatment of splenocytes with cadmium, reaction was done in a 15-mL tube. Treated cells (4-10 x 10^6) were washed twice with PBS, and lysed in 5 mM Tris buffer, pH 7.4, containing 0.5% Triton X-100 and 20 mM EDTA at 4°C for 20 min. After centrifugation at 28,000 x *g* for 20 min, DNA fragments were extracted with phenol-chloroform and precipitated in ethanol. The sample was treated with 20 µg/mL RNase A (Sigma Chemical Co.) and electrophoresed on a 1.2% agarose gel.

Cell fractionation and Western Blot Analysis

After treated with cadmium or recombinant TNF-α (Sigma Chemical Corp.), cells were collected by centrifuge at 600 x g for 5 min and homogenized in 20 mM Hepes, pH 7.8, containing 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, and protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride (Wako Co., Osaka, Japan), 5 µg/mL pepstatin (Peptide Institute Inc., Osaka, Japan), and 5 µg/mL leupeptin (Peptide Institute Inc.). The homogenates were kept in ice for 10 min. After centrifuge at 2,000 x g for 10 min, the supernatants were used as cytoplasmic fraction. The pellets were resuspended in 20 mM Hepes, pH 7.8, containing 0.42 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM dithoiothreitol, 25% glycerol, and protease inhibitors at 4°C for 30 min. Then, the samples were centrifuged at 25,000 x g for 20 min at 4°C and the clear supernatants were used as nuclear fractions. Protein concentrations were measured with a bicinchoninic acid kit (Pierce Chemical Corp., Rockford, IL, USA) using bovine serum albumin as a standard. Twenty to forty micrograms of proteins were subjected to 7.5-15% polyacrylamide gels containing 0.1% SDS under reducing conditions (Laemmli, 1970). Proteins in an SDS gel were electrophoretically transferred at 2 mA/cm^2 for 20 min onto Immobilon membrane (Millipore Corp., Osaka, Japan) in 25 mM Tris, 192 mM glycine, and 20% methanol with an Atto semi-dry horizontal electrophoretic transfer unit (Atto, Tokyo, Japan). The membrane was blocked with 8% casein in PBS containing 0.1% Tween 20 at room temperature for 3 hr. The transferred membrane was then incubated with monoclonal antibodies against Bcl-2 (Ishido *et al*., 1999, 2002), polyclonal antibodies against CAD (1:500 dilution; Millennium Biotech., Ramona, CA, USA), or monoclonal antibodies against PARP (2 µg/mL; Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA) in PBS containing 0.1% Tween 20 and 5% bovine serum albumin overnight at 4°C. After incubation with primary antibodies, the sheets were washed three times for 5 min each with PBS containing 0.1% Tween 20, and the antibodies were detected with horseradish peroxidase-conjugated secondary IgG using an Enhanced chemiluminescence (ECL) Western blotting detection kit according to the instructions of the manufacturer (Amersham

Pharmacia Biotech, Uppsala, Sweden). Gels were calibrated with prestained molecular markers (Bio-Rad, Hercules, CA, USA).

Isolation of human genomic DNA

Human genomic DNA was prepared from white blood cells obtained from normal volunteers following the method as described (DiLella and Woo, 1987).

Immunodepletion

Cell lysates of wild type LLC-PK_1 cells were prepared as above. The lysates (135-270 µg) were incubated with either 10 µL of control human serum or 10 µL of anti-CAD antibody (5 µg) for 16 hr at 4°C. Followed by immunoprecipitation with 10 µL of 50% suspension of Protein A/G PLUS agarose (Santa Cruz Biotechnology, Inc.), the immunodepleted cell lysates were diluted with equal amounts of 40 mM PIPES (pH 7.5), 200 mM NaCl, 0.2% CHAPS, 20% sucrose, 2 mM EDTA, and 20 mM dithiothreitol and were then mixed with human genomic DNA (2 µg) at 30°C for 2 hr in the presence of 200 ng of the recombinant active caspase-3 (MBL Corp., Nagoya, Japan). The reaction was terminated by the dilution with 7-fold 5 mM Tris buffer, pH 7.4, containing 0.5% Triton X-100 and 20 mM EDTA. After centrifugation at 10,000 x g for 10 min, the DNA fragments were extracted with phenol- chloroform and precipitated in ethanol. The samples were treated with 20 µg/mL RNase A and electrophoresed on a 1.5% agarose gel.

RESULTS

Cadmium (10 µM) elicited DNA fragmentation as early as 7 hr after exposure of LLC-PK_1 cells, which was detected with ethidium bromide staining of an agarose gel (Ishido *et al*., 1995). Since PARP is a proteolytic substrate for caspase-3, Western blot analysis was carried out to detect a cleavage product using apoptotic LLC-PK_1 cells induced by cadmium or recombinant human TNF-α. DNA fragmentation induced by 10 ng/mL of recombinant human TNF-α was observed on an ethidium bromide-stained gel as early as 24 hr after the addition of the cytokine into LLC-PK_1 cells (data not shown). Fig. 1 shows that the cleavage product was significantly evident after exposure to cadmium (lanes 2-4). They were cleaved by cadmium more than TNF-α did (lanes 5-7), indicating that activation of the protease by the metal was sufficient to cleave the biosubstrate.

We next examined the nuclear localization of CAD. During TNF-α (10 ng/mL)-induced apoptosis, CAD was detected in nuclear fractions at 16-24 hr (Fig. 2A, lanes 2,3). However, it was not seen during cadmium-induced apoptosis (Fig. 2A, lanes 12-16). Fig. 2B shows the lack of non-nuclear proteins, Bcl-2 in the nuclear fractions which contained CAD translocated by TNF-α seen in Fig. 2A, confirming the reliability of the quality of the nuclear-cytoplasmic split used. Thus, nuclear localization of CAD during renal apoptosis was differently regulated by the apoptotic stimuli, both of which could activate caspase-3-like protease (Sclegel *et al*., 1996; Kondoh *et al*. 2002; Lee *et al*., 2006).

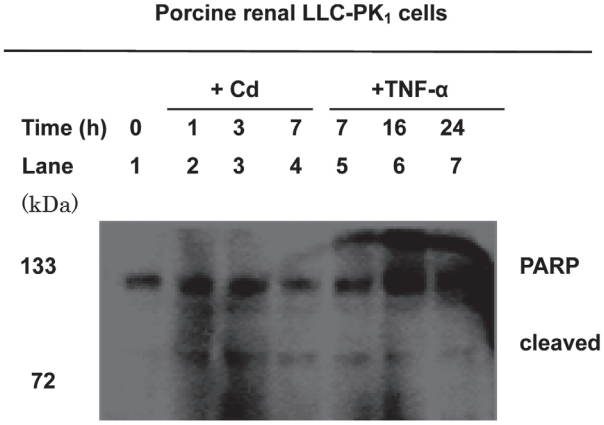

Fig. 1. Cadmium cleaves PARP as sufficiently as TNF-α does. LLC-PK_1 cells (4 x 10^6) were exposed to nothing (lane 1), 10 µM cadmium (lanes 2-4), or 10 ng/mL recombinant human TNF-α (lanes 5-7) for indicated periods. Nuclear fractions of cell lysates (30 µg) were subjected to a 7.5% SDS-gel electrophoresis and immunoblotted with monoclonal antibodies against PARP. The positions of size markers (kDa) are shown on the left margin.

Since cadmium also exerts its effects on the functions of immune system (Fujimaki *et al*., 2000), we investigated the apoptotic effects of cadmium on rat splenocytes. Figure 3A shows the formation of DNA fragments with time following the addition of cadmium (10 µM) into the rat primary splenocytes (1 x 10^7). As early as 7 hr after exposure of splenocytes to the metal, DNA fragments were clearly visible on an ethidium bromide-containing agarose gel (Fig. 3A, lane 3). The DNA fragmentation was still detectable at 22 hr (Fig. 3A, lane 4). Nuclear fractions from the splenocytes which were treated with 10 µM cadmium for 7 hr contained CAD (Fig. 3B, lane 2), indicating the nuclear translocation of CAD during cadmium-mediated apoptosis of splenocytes.

To examine the specificity of anti-CAD antibody employed in this study, we used cell-free extracts of wild type LLC-PK_1 cells. Incubation of the mock-depleted cell

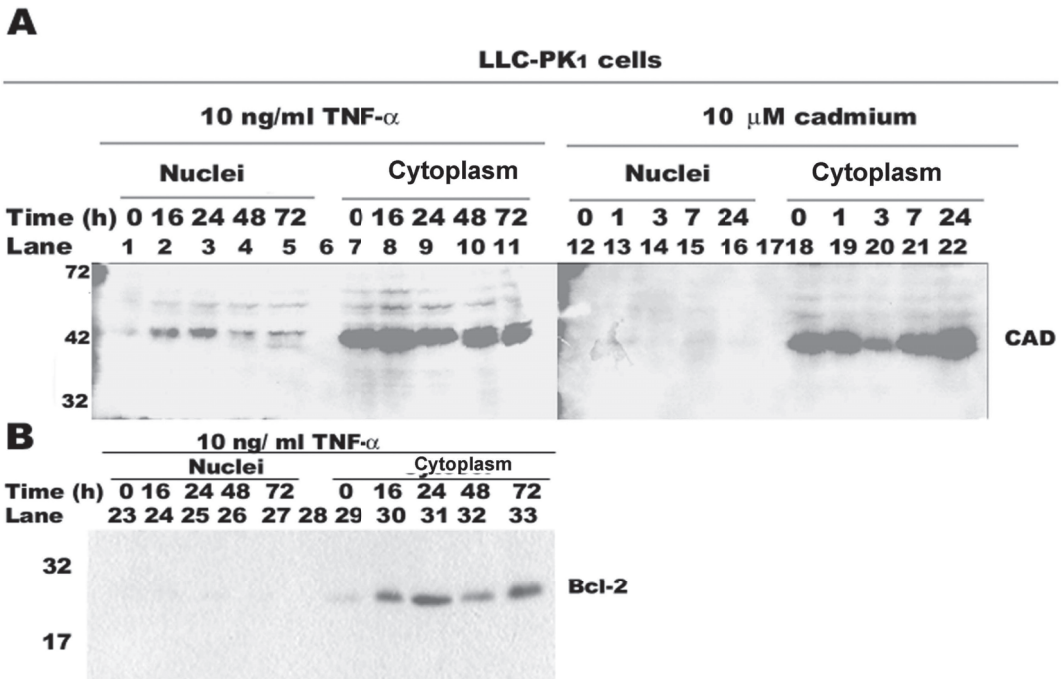

Fig. 2. Nuclear localization of CAD by TNF-α, but not by cadmium during their renal apoptosis. LLC-PK$_1$ cells (4 x 10^6) were treated with 10 ng/mL recombinant human TNF-α (lanes 1-5 and 7-11) or 10 μM cadmium (lanes 12-16 and 18-22) for indicated periods. After cell fractionation, 20 μg proteins of nuclear fractions or 30 μg proteins of cytoplasmic fractions were separated through an SDS-polyacrylamide gel and immunoblotted with polyclonal antibodies against CAD (*A*) or with monoclonal antibodies against Bcl-2 (*B;* lanes 23-27 and 29-33). The positions of size markers (kDa) are shown on the left margin.

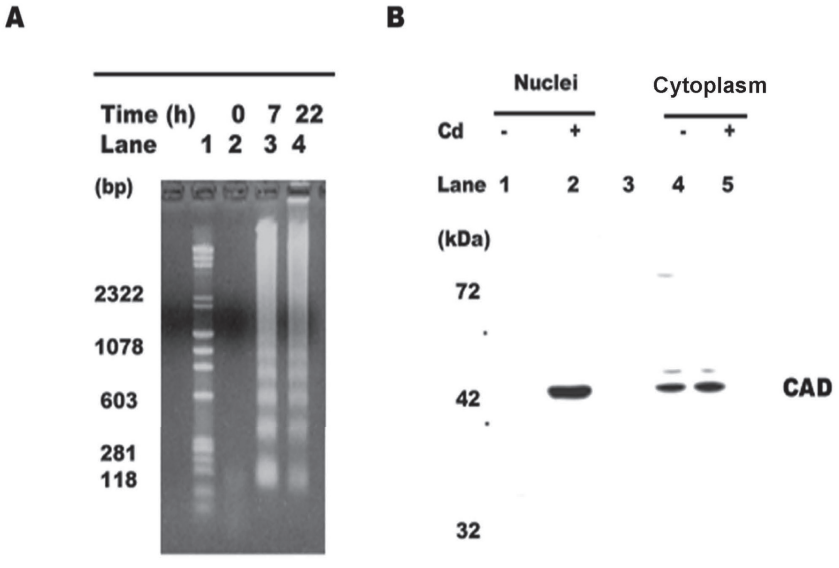

Fig. 3. Apoptotic actions of cadmium in rat splenocytes. Rat splenocytes (1 x 10^7) were treated with 10 μM cadmium for indicated periods. Treated cells were analysed for DNA fragmentation (*A*), and nuclear localization of CAD (*B*) as described in 'MATERIALS AND METHODS'. Forty micrograms of proteins of both nuclear fractions and cytoplasmic fractions were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with polyclonal antibodies against CAD (*B*). The positions of size markers (kDa) are shown on the left margin.

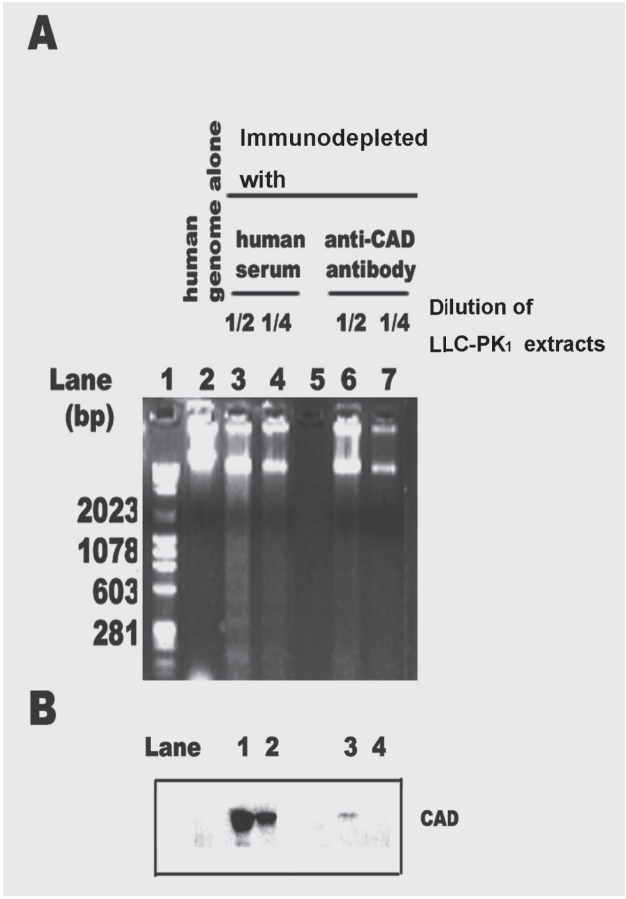

Fig. 4. Immunodepletion of CAD by anti-CAD antibody results in remarkable decrease to induce DNA fragmentation in the presence of the recombinant active caspase-3 in porcine kidney LLC-PK_1 cell extracts. *A;* Mock-(lanes 3 and 4) and CAD-depleted (lanes 6 and 7) extracts were incubated with human genomic DNA (2 µg) in the presence of the recombinant active caspase-3 at 30°C for 2 hr. The DNA fragments were extracted as described in 'MATERIALS AND METHODS'. A half of dilution of the extracts (lanes 3 and 6) had 270 µg proteins. *B;* Mock-(lanes 1 and 2) and CAD-depleted (lanes 3 and 4) extracts (30 µg proteins for lanes 1 and 3 and 15 µg proteins for lanes 2 and 4) were subjected to a 10% SDS-polyacrylamide electrophoresis, followed by Western blot analyses, using anti-CAD antibody.

extracts (270 µg) with human genomic DNA in the presence of active caspase-3 at 30°C for 2 hr induced DNA fragmentation (Fig. 4A, lane3). It was slightly detectable in one fourth of the original concentration of the cell extracts (135 µg; Fig. 4A, lane4). However, CAD-depleted cell extracts (135-270 µg) resulted in remarkable decrease in the induction of DNA fragmentation in the presence of active caspase-3 (Fig. 4A, lanes 6 and 7). Western blot analyses revealed that CAD in the cell extracts was largely depleted with anti-CAD antibody (Fig. 4B). Thus, immunodepletion experiments confirmed the specificity of the anti-CAD antibody used in Western blotting analyses (Figs. 2 and 3).

DISCUSSION

In this study, we demonstrate that cadmium did not allow to induce the translocation of CAD into the nuclei in porcine kidney LLC-PK_1 cells where TNF-α did, and that in rat splenocytes, same cadmium could allow CAD to enter the nuclei. These data suggest that nuclear localization of CAD during apoptotsis by same apoptotic stimuli is regulated in different cell types (*e.g.* apoptosis by cadmium in LLC-PK_1 cells and splenocytes), and that apoptotic stimuli were also regulated to allow the nuclear localization of CAD in a single cell (*e.g.* apoptosis by cadmium and TNF-α in LLC-PK_1 cells).

The activated caspase3 could cleave PARP in renal cells and could lead to nuclear localization of CAD in splenocytes. However, we could not exclude the possibility that activated caspase cascade shown here might be concomitant with the other unknown death signaling pathway of the metal. It was reported that direct modification of cysteine residues of caspase-3 by $HgCl_2$, one of an apoptosis-inducing metal in LLC-PK_1 cells (Duncan-Achanzar *et al*., 1996), led to the inhibition of the protease activity (Kim *et al*., 1997.) Therefore, activation of caspase-3-like protease by exposure of the cells to cadmium may be indirect effect of the metal. Because of this possibility, free cadmium ion may be much less than 10 µM in the cells, leading to indirectly activate the caspase protease with less degree than expected.

Further, although CAD is also rich in cysteine residues (Enari *et al*., 1998; Mukae *et al*., 1998), it remains unknown whether these residues were modified by exposure of the cells to cadmium.

Northern blot analyses revealed that the expression of CAD mRNA was limited and that the pattern of expression of CAD mRNA was different between species (Mukae *et al*., 1998). Overexpression of CAD facilitated DNA fragmentation in HeLa cells (Mukae *et al*., 1998). These data suggested that the CAD expression level can determine the ability of cells to undergo DNA fragmentation. However, this was not case. Susceptibility of DNA fragmentation of both LLC-PK_1 cells and rat splenocytes by cadmium seems to be same as judged by kinetics of DNA fragmentation analyses: the amounts of cytoplasmic CAD in rat splenocytes were much less than those in LLC-PK_1 cells (Fig. 2A versus Fig. 3B). The result that

cadmium caused DNA fragmentation in LLC-PK_1 cells without nuclear CAD indicates that the level of CAD expression was not a sole determinant of susceptibility of DNA fragmentation.

From the results in this study, a few questions were raised: what is an effctor executioner, distinct from CAD, which is activated by cadmium-induced caspase-3-like proteolysis in renal cells? What is a major death signaling pathway of cadmium, which will be coupled to a caspase-3-like proteolysis cascade? How is the nuclear localization of CAD regulated during programmed cell death of a single cell? These are under investigation in our laboratory.

ACKNOWLEDGMENTS

We thank Prof. Tsuguyoshi Suzuki for encouragement.

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

REFERENCES

Azzouzi, B.E., Tsangaris, G.T., Pellegrini, O., Manuel, Y., Benveniste, J. and Thomas, Y. (1994): Cadmium induces apoptosis in a human T cell line. Toxicology, **8**, 127-139.

Cohen, G.M. (1997): Caspases: the executioners of apoptosis. Biochem. J., **326**, 1-16.

DiLella, A.G. and Woo, S.L.C. (1987): Cloning large segments of genomic DNA using cosmid vectors. Methods Enzymol., **152**, 199-212.

Duncan-Achanzar, K.B., Jones, J.T., Burke, M.F., Carter, D.E. and Laird, H.E. (1996): Inorganic mercury chloride-induced apoptosis in the cultured porcine renal cell line LLC-PK_1 cells. J. Pharmacol. Exp. Ther., **277**, 1726-1732.

Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A. and Nagata, S. (1998): A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature, **391**, 43-50.

Friberg, L., Kjellstrom, T., Nordberg, G.F. and Piscator, M. (1979): Cadmium, in Handbook on the Toxicolgy of Metals (Friberg, L., Nordberg, G.F. and Vouk, V.B. eds.) pp.355-381, Elsevier/North-Holland Biomedical Press, Amsterdam.

Fujimaki, H., Ishido, M. and Nohara, K. (2000): Induction of apoptosis in mouse thymocytes by cadmium. Toxicol. Lett., **115**, 99-105.

Graham, F.L. and Van der Eb, A.J. (1973): A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology, **52**, 456-467.

Haecker, G. and Vaux, D.L. (1994): Viral, worm, and radical implications for apoptosis. Trends Bichem. Sci., **19**, 99-100.

Halenbeck, R., MacDonald, H., Roulston, A., Chen, T.T., Contoy, L. and Williams, L.T. (1998): CPAN, a human nuclease regulated by the caspase-sensitive inhibitor DFF45. Curr. Biol., **8**, 537-540.

Ishido, M., Homma, S., Leung, P. and Tohyama, C. (1995): Cadmium-induced DNA fragmentation is inhibitable by zinc in porcine kidney LLC-PK_1 cells. Life Sci., **56**, PL351-PL356.

Ishido, M., Homma, S., Tohyama, C. and Suzuki, T. (1998): Apoptosis in rat renal proximal tubular cells induced by cadmium. J. Toxicol. Environ. Health, **55**, 101-112.

Ishido, M., Tohyama, C. and Suzuki, T. (1998): c-Myc is not involved in cadmium-elicited apoptotic pathway in porcine kidney LLC-PK_1 cells. Life Sci., **63**, 1195-1204.

Ishido, M., Tohyama, C. and Suzuki, T. (1999): Cadmium-bound metallothionein induces apoptosis in rat kidneys, but not in cultured kidney LLC-PK_1 cells. Life Sci., **64**, 797-804.

Ishido, M., Suzuki, T., Adachi, T. and Kunimoto, M. (1999): Zinc stimulates DNA synthesis during its anti-apoptotic action independently with increments of an anti-apoptotic protein Bcl-2 in porcine kidney LLC-PK_1 cells. J. Pharmacol. Exp. Ther., **290**, 923-928.

Ishido, M., Ohtsubo, R., Adachi, T. and Kunimoto, M. (2002): Attenuation of both apoptotic and necrotic actions of cadmium by Bcl-2. Environ. Health Perspect., **110**, 37-42.

Kerr, J.F.R., Wyllie, A.H. and Currie, A.R. (1972): Apoptosis: a basic biologocal phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer, **26**, 239-257.

Kim, Y.-M., Talanian, R.V. and Billiar, T.R. (1997): Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J. Biol. Chem., **272**, 31138-31148.

Kondoh, M., Araragi, S., Sato, K., Higashimori, M., Takiguchi, M. and Sato, M. (2002): Cadmium induces apoptosis partly via caspase-9 activation in HL-60 cells. Toxicology, **170**, 111-117.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**, 680-685.

Lee, W.-K., Abouhanmed, M. and Thevenod, F. (2006): Caspase-dependent and -independent pathways for cadmium-induced apoptosis in cultured kidney proximal tubule cells. Am. J. Renal Physol., **291**, F823-F832.

Liu, G., Zou, H., Luo, T., Long, M., Bian, J., Liu, X., Gu, J., Yuan, Y., Song, R., Wang, Y., Zhu, J. and Liu, Z. (2016): Caspase-dependent and caspase-independent pathways are involved in cadmium-induced apoptosis in primary rat proximal tubular cell culture. PlosOne, **11**, e0166823.

Liu, X., Zou, H., Slaughter, C. and Wang, X. (1997): DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell, **89**, 175-184.

Liu, X., Li, P., Widlak, P., Zou, H., Luo, X., Garrard, W.T. and Wang, X. (1998): The 40-kDa subunit of DNA fragmentation factor induces DNA fragmentation and chromatin condensation during apoptosis. Proc. Natl. Acad. Sci., **95**, 8461-8466.

Mukae, N., Enari, M., Sakahira, H., Fukuda, Y., Inazawa, J., Toh, H. and Nagata, S. (1998): Molecular cloning and characterization of human caspase-activated DNase. Proc. Natl. Acad. Sci., **95**, 9123-9128.

Nagata, S. (1997): Apoptosis by death factor. Cell, **88**, 355-365.

Nordberg, M. and Nordberg, G.F. (2016): Trace element research-historical and future aspects. J. Trace Elem. Med. Biol., **38**, 46-52.

Nicholson, D.W. and Thornberry, N.A. (1997): Caspases: killer proteases. Trends Biochem. Sci., **22**, 299-306.

Perry, D.K., Smyth, M.J., Stennicke, H.R., Salvesen, G.S., Duriez, P., Poirier, G.G. and Hannum, Y.A. (1997): Zinc is a potent inhibitor of the apoptotic protease, caspase-3. J. Biol. Chem., **272**, 18530-18533.

Sakahira, H., Enari, M. and Nagata, S. (1998): Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature, **391**, 96-98.

Sakahira, H., Enari, M. and Nagata, S. (1999): Functional differences of two forms of the inhibitor of caspase-activated DNase,

ICAD-L, and ICAD-S. J. Biol. Chem., **274**, 15740-15744.
Salvesen, G.S. and Dixit, V.M. (1997): Caspases: intracellular signaling by proteolysis. Cell, **91**, 443-446.
Sclegel, J., Peters, I., Orrenius, S., Miller, D.K., Thornberry, N.A., Yamin, T.-T. and Nicholson, D.W. (1996): CPP32/Apopain is a key interleukin 1β converting enzyme-like proteases involved in Fas-mediated apoptosis. J. Biol. Chem., **271**, 1841-1844.
Sun, X.-M., MacFarlane, M., Zhung, J., Wolf, B.B., Green, D.R. and Cohen, G.M. (1999): Distinct caspase cascade are initiated in receptor-mediated and chemical-induced apoptosis. J. Biol. Chem., **274**, 5053-5060.
Thevenod, F. and Lee, W.-K. (2013): Cadmium and cellular signaling cascades: interactions between cell death and survival pathways. Arch. Toxicol., **87**, 1743-1786.
Thornberry, N.A. and Lazebnik, Y. (1998): Caspases: enemies within. Science, **281**, 1312-1316.
Wolf, B.B. and Green, D.R. (1999): Suicidal tendencies: apoptotic cell death by caspase family proteinases. J. Biol. Chem., **274**, 20049-20052.
Wylle, A.H. (1980): Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature, **284**, 555-556.
Xu, C., Johnson, J.E., Singh, P.K., Jones, M.M., Yan, H. and Carter, C.E. (1996): *In vivo* studies of cadmium-induced apoptosis in testicular tissue of the rat and its modulation by a chelating agent. Toxicology, **107**, 1-8.
Yan, H., Carter, C.E., Xu, C., Singh, P.K., Jones, M.M., Johnson, J.E. and Dietrich, M.S. (1997): Cadmium-induced apoptosis in the urogenital organs of the male rat and its suppression by chelation. J. Toxicol. Environ. Health, **52**, 149-168.
Zhang, J., Liu, X., Scherer, D., Kaer, L.V., Wang, X. and Xu, M. (1998): Resistance to DNA fragmentation and chromatin condensation in mice lacking the DNA fragmentation factor 45. Proc. Natl. Acad. Sci., **95**, 12480-12485.