— **Minireview** —

Regulation of Cell Fate by Cadmium and Zinc

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Effects of the heavy metals cadmium and zinc on the regulation of cell destiny are reviewed in light of an apoptogenic metal of cadmium and the antiapoptotic nature of zinc. Exposure of renal cells to cadmium causes apoptotic features, DNA fragmentation and chromatin condensation in earlier stages of cadmium cytotoxicity than the cadmium- induced necrotic phase. The molecular mechanism of cadmium-induced apoptosis is poorly understood. Neither cadmium-metallothionein nor an immediate early gene such as c-myc is involved in the apoptotic pathway. In contrast, zinc can abolish cadmium-induced apoptosis. Although the mechanism underlying zinc inhibition of apoptosis remains uncertain, one possibility is that the ability of zinc to facilitate DNA synthesis might contribute to its protective effect on apoptosis.

Key words —— apoptosis, cadmium, zinc

Cadmium is a nephrotoxic metal.[1] The proximal tubule of the mammalian kidney is a major target of chronic cadmium-induced toxicity.[2] Cadmium chloride induces the synthesis of metallothionein in the liver, which, when released into circulation, is taken up by renal tubule cells.[3] In the kidney, cadmium associated with metallothionein is degraded in lysosomal compartments, and the released cadmium in turn stimulates a cascade of toxic effects.[4] The development of cadmium-induced lesions in the kidney is characterized by proteinuria and excessive urinary excretion of other substrates such as enzymes, amino acids, and glucose.[1]

Necrosis is the mode of cell death reported to be exerted by cadmium, which disrupts energy producing systems.[5] Cellular membrane defects and an increased influx of calcium ions have been observed at the end-point of toxicity of the metal.[6]

Recently, the apoptogenic nature of cadmium was demonstrated in experiments involving DNA fragmentation and chromatin condensation in porcine renal cultured LLC-PK_1 cells (Fig. 1A and B; Ref. 7) and in rat renal proximal tubular cells (Fig. 1C and D; Refs. 8, 9). In addition, apoptosis has been observed in the human T cell hybridoma,[10] rat urogenital organs,[11] rat liver,[12] and immunocytes.[13,14] Apoptosis occurs before necrotic processing.[7,14,15] This apoptosis may facilitate a suicidal mechanism programmed to shut down the cellular metabolism in cells affected by the metal before healthy cells undergo cell death. Thus, the apoptotic processing *per se* functions as a cellular defense in the early stages of cadmium cytotoxicity.

Oncogenes in Apoptotic Responses to Cadmium Cytotoxicity

Exposure to cadmium evokes in cells a number of responses that involve not only death signaling reactions, but also cellular protective reactions against the toxicity;[5,7,15–19] for this reason, it has proven difficult to identify the signal pathway of the metal in the processes of cell death. Glutathione plays the role of a first line of defense against cadmium cytotoxicity.[16,17] Intracellular cadmium ions, which escape sulfhydryl reaction with glutathione, exert numerous cellular responses, including the induction of proto-oncogenes[18] and heat shock proteins.[19] Ultimately, free cadmium ions are sequestered by the chemical reaction with metallothionein, which is inducible at a later phase of cadmium exposure.[15] Cell fate is dependent on which reaction exceeds the others.

There is evidence that the activation of proto-

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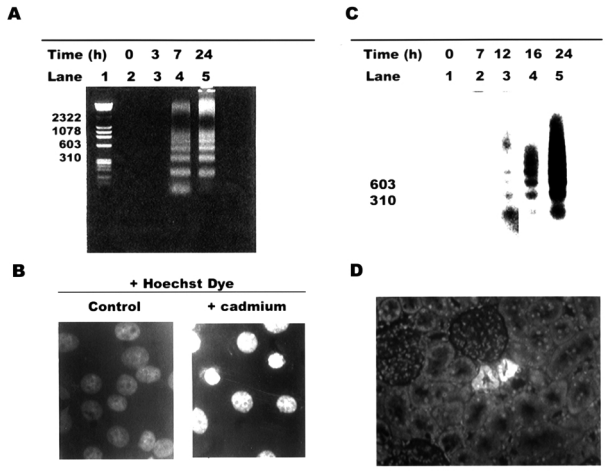

Fig. 1. Cadmium Induces Apoptosis in Cultured Porcine Kidney LLC-PK$_1$ Cells (*A* and *B*) and Rat Kidneys (*C* and *D*)

A: DNA fragmentation occurs in cadmium chloride (10 μM)-exposed LLC-PK$_1$ cells. B: Hoechst dye-staining shows chromatin condensation in cadmium chloride (10 μM)-exposed LLC-PK$_1$ cells. C: Kinetics of formation of DNA ladders in Cd-MT (0.15 mg per kg body weight)-treated rat kidneys. D: *In situ* detection of apoptotic cells in renal proximal tubular cells from Cd-MT (0.15 mg per kg body weight)-treated rat.

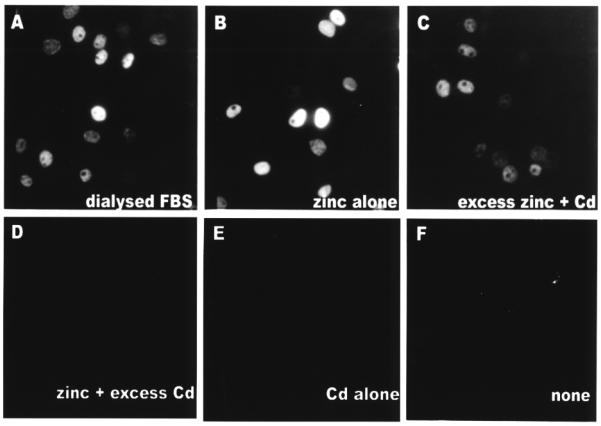

Fig. 2. Stimulation of BrdU Incorporation by Zinc

Porcine kidney LLC-PK$_1$ cells were exposed to 1% dialysed FBS (A), 5 μM zinc alone (B), 5 μM zinc plus 1 μM cadmium (C), 0.1 μM zinc plus 1 μM cadmium (D), 1 μM cadmium alone (E), and none (F). Then, they were pulse-labeled with 10 μM BrdU. Original magnification: 200 ×.

oncogenes is involved in apoptotic events.[20] Cadmium activates c-myc transcripts before the metal induces DNA fragmentation.[21] This finding indicates that an apparent correlation exists between the induction of c-myc transcripts and the promotion of apoptosis. Therefore, we investigated whether c-myc is a necessary component of cadmium-induced apoptosis.[21] Even when actinomycin D was used to block transcriptional activation, or antisense oligodeoxynucleotide complementary to c-myc was used to block translation of the protein, cadmium was still able to induce apoptosis. These results suggest that the metal elicits apoptosis by a mechanism other than the regulation of c-myc expression: the transcriptional activation of c-myc is not always involved in cell death events.[21]

Cellular commitment to apoptosis appears to precede the activation of the c-myc molecule.[21] This leads to the following line of questioning, namely, defining the role of c-myc activation during metal-induced apoptosis. c-Myc acts as a double-edged sword, because it is both capable of driving a cell through the cell cycle under certain conditions, but it can also provoke cell death under other conditions.[22] During cadmium-induced apoptosis, bromodeoxyuridine (BrdU) incorporation was not facilitated (Fig. 2E), indicating that the activation of c-myc by the metal was not a consequence of DNA repair or DNA synthesis. Identification of the target molecule of c-myc activated by cadmium will reveal the physiological significance of the oncogene in apoptosis.

Other proto-oncogenes such as c-fos, c-jun, and p53 have also been reported to be activated by cadmium.[23,24] Actinomycin D might be expected to block the transcriptional activation not only of c-myc, but also of c-fos, c-jun, and p53. It is of note that cadmium enhances its cytotoxicity in fibroblasts which lack c-fos;[25] activation of the relevant genes may protect cells from cadmium toxicity.

Cadmium-Metallothionein (Cd-MT) in Cadmium-Induced Apoptosis

Recently, it was reported that cadmium-associated metallothionein (Cd-MT) facilitates cadmium-induced apoptosis in human kidney 293 cells.[26] However, in the study of cultured porcine kidney LLC-PK$_1$ cells, the level of expression of Cd-MT was observed to be extremely low at a time when apoptosis was already detectable.[15] We examined the effects of intracellular MT (1.9 μg/ 2×10^6 cells), the level of which was similar to that induced by zinc (25 μM; 24 hr), on cadmium-induced apoptosis.[27] Intracellular Cd-MT did not facilitate cell death. Furthermore, the addition of purified Cd-MT to the cultured LLC-PK$_1$ cells failed to induce apoptosis. It remains unclear whether this discrepancy comes from a difference in the amount of cadmium ions liberated from metallothionein or the use of different cell lines. Therefore, the notion that Cd-MT promotes cadmium-induced apoptosis *in vitro* remains a subject of controversy.

In contrast, the administration of purified Cd-

MT (0.15 mg MT bound Cd per kg body weight) to the rat induced apoptosis of the kidney at 12 hr (Fig. 1C and D; Ref. 8). It was still detected 24 hr after administration. The induction of apoptosis by Cd-MT was specific to the kidney; it was not observed in the cerebrum, cerebellum, heart, lung, liver, testis, dorsolateral prostate, or ventral prostate.[27] To characterize the specific types of renal cells involved in DNA fragmentation, a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique was applied to histological sections of the kidney.[8] The kidneys from rats treated with Cd-MT for 12 hr displayed labeling of proximal tubular cells that was significant (Fig. 1D). Chromatin condensation, another feature of apoptosis, was also observed in the proximal tubular cells.[8]

Thus, Cd-MT induces apoptosis in rat kidneys, but not in cultured renal cells, suggesting that the ionic form of cadmium is required for programmed cell death.

The Role of Zinc in Antiapoptotic Actions

It has been believed that DNA fragmentation, which is defined as one feature of apoptosis in the final stage of the apoptotic process, may be achieved by calcium/magnesium-dependent endonuclease.[28] Since the enzyme requires calcium or magnesium for its activation,[28] zinc is thought to prevent apoptosis by inhibiting such enzyme activation. However, the observation that cadmium, a divalent ion, induced apoptosis in intact renal cells, and that it was inhibitable by zinc at a micromolar concentration,[7] raises a couple of questions. Firstly, given that the target for the antiapoptotic action of zinc was presumed to be nuclear endonuclease, how do cadmium and zinc regulate the cell destiny? Cadmium induces apoptosis; whereas zinc prevents it, although both cadmium and zinc are able to inhibit the activity of the endonuclease of isolated nuclei.[29] Secondly, a micromolar concentration of zinc was able to inhibit cadmium-induced apoptosis, whereas a millimolar concentration of zinc was required for the inhibition of purified calcium-dependent endonuclease.[30] These observations suggest the existence of another mechanism for the protective effect of zinc on programmed cell death.

A number of peptide factors protect cells from apoptosis, including neurotrophins, cytokines and growth factors such as IGF-1 and PDGF.[31] These factors drove cells to proliferate with various degrees of cell growth,[31] suggesting that the induction of cell proliferation protects cells against apoptosis. Therefore, the hypothesis that zinc has mitogenic activity that contributes to its inhibitory effect on apoptotic cell death was examined.[32] Stimulation of cultured porcine kidney LLC-PK_1 cells with dialyzed FBS (1%) and zinc (5 μM) facilitated DNA synthesis to a greater extent than seen in the unstimulated control cells (Fig. 2). A molar excess of zinc (5 μM) could still promote BrdU incorporation in the presence of cadmium (1 μM). However, a lower concentration of zinc (0.1 μM) than of cadmium (1 μM) failed to facilitate BrdU incorporation; therefore, cadmium induced apoptosis. Thus, it was suggested that the ability of zinc to facilitate DNA synthesis might contribute to its protective effect on apoptosis.

To confirm this, we next used dialysed FBS as a mitogen to examine whether cadmium-induced apoptotic cell death was inhibited by mitogenic factors in serum instead of by zinc.[32] Ten percent of dialysed FBS completely inhibited 1 μM cadmium-induced DNA fragmentation. One percent of dialysed FBS was also sufficient to inhibit it in correlation with the extent of the cell growth by 1% dialysed FBS in the presence of 1 μM cadmium. DNA fragmentation was not seen in the absence of cadmium. Thus, cadmium-induced nephroptosis was inhibitable by zinc, and by any growth factors in serum, both of which induce cell growth, indicating that the inhibition of programmed cell death by zinc occurs via its activity in promoting the entry of cells into the S phase of DNA synthesis of the cell cycle.

The characterization of signal transduction pathways activated by peptide factors has led to the identification of critical mediators of cell survival. So far, the best-characterized mediators of cell survival are the bcl-2 family[33] and phosphoinositide 3-kinase (PI3-kinase), and its downstream target, Akt.[34] IGF-1 and insulin suppressed apoptosis via PI3-kinase and the Akt pathway without affecting the expression levels of Bcl-2. In contrast, zinc increased the level of Bcl-2.[32] However, zinc did not seem to activate PI3-kinase or Akt in cultured renal cells. It was observed that cultured cerebellar granule neurons die by apoptosis when switched from a medium containing an elevated concentration (25 mM) of potassium (K^+) to one with a lower concentration of K^+ (5 mM), and that an elevated concentration of K^+, the signaling pathway of which was PI3-kinase-independent, maintained cell surviva.[35] These observations suggest the existence of another survival signaling pathway, and it is unknown whether the inhibition of apoptosis by both ions is mediated through a common mechanism. Identification of putative

growth factors in serum, which contribute to the inhibition of cadmium-induced apoptosis, will help to reveal the molecular mechanism of the antiapoptotic action of zinc.

CONCLUSION

The recent discovery of the apoptogenic nature of cadmium has resulted in the development of a new paradigm for a protective mechanism, leading to the identification of a new role of zinc in antiapoptotic actions. On the other hand, the mechanism underlying the death signaling of cadmium still remains uncertain. The well-known apoptotic pathway follows a combination of proteases and an endonuclease, caspases and their activated nuclease.[36,37] It is tempting to investigate the involvement of these in cadmium-mediated apoptosis.

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