Current Topics

Advances in Biology and Toxicology of Environmental Metals/Metalloids

Cadmium Renal Toxicity *via* Apoptotic Pathways

Yasuyuki Fujiwara,[a] Jin-Yong Lee,[a] Maki Tokumoto,[a,b] and Masahiko Satoh*,[a]

[a] *Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University; 1–100 Kusumoto-cho, Chikusa-ku, Nagoya 464–8650, Japan: and*
[b] *Laboratory of Chemical Toxicology and Environmental Health, Showa Pharmaceutical University; 3–3165 Higashi-Tamagawagakuen, Machida, Tokyo 194–8543, Japan.*
Received July 30, 2012

Cadmium is a nonessential heavy metal and ubiquitous potential environmental pollutant. Although the kidney proximal tubule is an important target for cadmium, the underlying cellular mechanisms of cadmium-induced renal toxicity remain elusive. Numerous studies have demonstrated that cadmium induces apoptotic cell death in various cell types *via* several apoptotic pathways, including mitochondria-mediated apoptotic cell death. In the epithelial cells of renal proximal tubules, cadmium can also induce apoptotic cell death *in vivo* and *in vitro*, which suggests that cell death of the epithelial cells through the apoptotic pathways is one of the key events in cadmium-induced renal toxicity. In this review, based upon the major findings of previous reports related to cadmium and apoptotic cell death, especially in the kidney and kidney proximal tubular cells, we present evidence for the current mechanisms of cadmium-induced renal toxicity *via* apoptotic cell death.

Key words cadmium; renal toxicity; apoptosis; renal tubular epithelial cell

1. INTRODUCTION

Cadmium is a nonessential divalent metal ion and induces severe toxic effects in multiple organs, such as the kidney, liver, and lung, in both humans and experimental animals. Moreover, renal toxicity caused by chronic cadmium exposure is well known.[1–3] Cadmium-induced renal toxicity has been observed in settings of industrial exposure and environmental pollution. Because cadmium has a long biological half-life (*i.e.*, 10–30 years), prolonged low-level exposure causes excessive accumulation in the body, especially in the kidney. The target sites of toxicity in the kidney are the S_1 and S_2 segments of the proximal tubules,[4] which are responsible for renal failure. Cellular and molecular mechanisms of cadmium-induced renal toxicity have been extensively studied. However, causal factors for the cellular mechanisms involved in cadmium-induced renal toxicity remain elusive.

Necrosis and apoptosis are two major types of cell death, and a variety of noxious substances and chemicals may induce different types of cell death depending on the mode of delivery, concentration, and cell types. Apoptosis is the mechanism by which cells are physiologically eliminated in metazoan organisms, and it plays an essential role, such as in regulating development and immune response and in clearing redundant or abnormal cells in organisms.[5,6] Apoptosis disorders induce a disruption of tissue homeostasis and functions and are associated with many diseases, such as cancer, neurodegenerative disease and toxin-induced disease.[7] Cadmium can induce apoptosis in various tissues and cultured cells, including mouse liver,[8] rat kidney and testis,[9–13] human T cell line (CEM-C12 cells), lymphoma U937 cells, normal hepatocytes, liver L-02 cells, fetal lung fibroblast MRC-5 cells and prostate epithelial cells,[14–19] mouse mesangial cells,[20] rat lung epithelial cells, cortical neurons and renal tubular epithelial cells,[21–23] and porcine kidney LLC-PK_1 cells.[24] These observations indicate that cell death of the proximal tubule cells in the kidney through apoptotic pathways is one of the key events in cadmium-induced renal toxicity. In this article, we will review recent developments related to cadmium-induced apoptosis in the kidney and cultured kidney-derived cells based upon the major findings of previous reports and our findings, and we will discuss the current mechanisms of cadmium-induced renal toxicity *via* apoptotic cell death.

2. ENDOPLASMIC RETICULUM-MEDIATED PATHWAY

2.1. Unfolded Protein Response Endoplasmic reticulum (ER) stress is involved in the induction of apoptosis and is characterized by the unfolded protein response (UPR).[25,26] A number of pathophysiological conditions, such as hypoxia and ischemia, viral infection, and neurodegenerative disorders, cause the accumulation of unfolded or misfolded proteins in the ER and cause ER stress, which induces a coordinated adaptive program called UPR.[27,28] The UPR acts to alleviate ER stress by increasing the folding capacity by upregulation of chaperon protein expression, inhibition of general protein translation, and activation of ER-associated degradation to promote the degradation of unfolded or misfolded proteins through the ubiquitin (Ub)–proteasome system. However, when the UPR is unable to rescue cells, ER stress induces apoptosis.

* To whom correspondence should be addressed. e-mail: masahiko@dpc.agu.ac.jp

In porcine renal proximal tubular epithelial LLC-PK_1 cells, cadmium has been demonstrated to induce apoptosis through ER stress.[29,30] Three major transducers for sensing ER stress are identified in the ER as follows: RNA-dependent protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1). As shown in Fig. 1, among these three pathways, the ATF6 pathway and IRE1 pathway contribute to apoptosis *via* the induction of CCAAT/enhancer-binding protein-homologous protein (CHOP) and *via* the activation of X-box binding protein 1 (XBP1) and phosphorylation of c-jun N-terminal kinase (JNK), respectively.[29,30] These *in vitro* observations suggest that cadmium causes renal toxicity through ER stress-mediated apoptosis. However, it is unclear whether the chronic and low-level exposure of mice to cadmium causes renal epithelial cell apoptosis *via* ER stress *in vivo*. Because the kidney is the tissue most affected by chronic cadmium toxicity, further *in vivo* studies are required to determine the importance of the ER stress pathways in cadmium-induced epithelial cell apoptosis and renal toxicity.

2.2. Calcium Release from the ER Calcium is an intracellular signal transmitter responsible for controlling cellular processes, including cell proliferation, differentiation, and survival/death.[31] Cadmium can elevate cytosolic calcium levels in renal tubular cells. Although the mechanism by which cadmium-induced cytosolic calcium elevation is unclear, this mechanism may be related to the release of calcium from an intracellular store, such as the ER, and to calcium uptake. The subsequently elevated cytosolic calcium initiates apoptosis in the renal tubular cells *via* the calpain-caspase pathway[32,33] (Fig. 2).

Phospholipase C (PLC) produces inositol-1,4,5-triphosphate (IP_3) from phosphatidylinositol 4,5-bisphosphate to induce the release of calcium from the ER *via* an interaction with IP_3 receptors.[34] Recently, in human embryonic kidney HEK 293 cells, the possible role of the PLC-calcium-dependent pathway in cadmium-induced apoptosis was demonstrated based on the following findings that a PLC-specific inhibitor, U73122, prevents the cadmium-dependent increase in cytosolic calcium levels and abolishes cadmium-induced calpain and caspase-3 activation.[35] Moreover, cadmium can activate a G-protein coupled receptor (GPCR) on the cell surface, which is followed by PLC activation,[32] and can increase cytosolic calcium levels *via* PLC activation in renal distal epithelial A6 cells.[36] These observations suggest that GPCR-mediated PLC activation may be one of the pathways in which cadmium releases calcium from the ER in renal proximal tubular cells (Fig. 2). However, cadmium-induced apoptosis was not fully prevented by U73122 pretreatment,[35] which indicates that cadmium also induces apoptosis through a PLC-independent pathway.

Sphingolipids, such as ceramide, play an important role in several cellular processes, including cell death and survival in various tissues.[37] As shown in Fig. 2, ceramide is known to function as a second messenger in cadmium-induced renal tubule epithelial cell apoptosis *via* the calpain-caspases pathway.[32,38–40] Exogenous *N*-hexanoyl-D-erythro-sphingosine (C_6-ceramide) administration increases cytosolic calcium levels and activates calpains and caspase-3 in rat kidney proximal tubule WKPT-0293 C1.2. cells.[39] Moreover, the inhibition of *de novo* ceramide synthesis using a ceramide synthase inhibitor abolishes calpain activation and apoptotic cell death that is

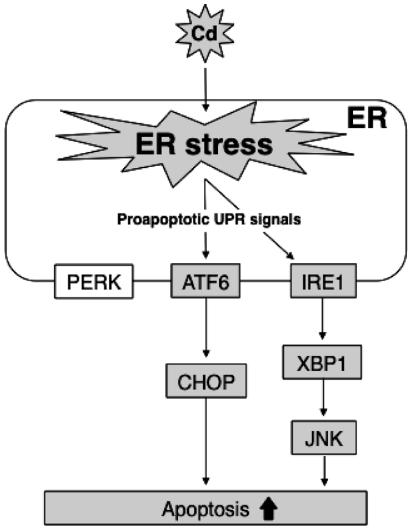

Fig. 1. Model for Cadmium-Induced Proapoptotic UPR Signals in the Kidney

Cadmium induces ER stress and causes the activation of UPR-dependent apoptotic pathways, such as the ATF6-CHOP pathway and IRE1-XBP1-JNK pathway.

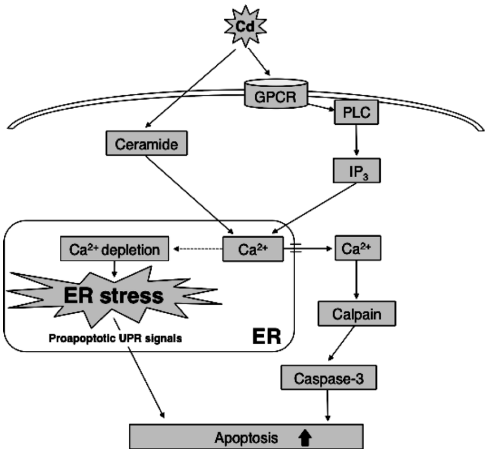

Fig. 2. Model for Cadmium-Induced Apoptosis *via* Calcium Release from the ER in the Kidney

Cadmium induces ceramide formation and consequently induces calcium release from the ER and calpain-caspase-3-mediated apoptosis. Cadmium also induces PLC activation, most likely *via* GPCR, which is followed by IP_3-mediated cytosolic calcium elevation and calpain activation.

caused by acute cadmium exposure in the cells.[38,39] Together, these findings demonstrate that cadmium can also cause an increase in cytosolic calcium levels, which may activate calpain and caspases through an increase in ceramide formation (Fig. 2). Moreover, because depletion of the calcium store in the ER may be related to ER stress,[41] it is likely that cadmium induces the release of calcium from the ER, which may cause not only the induction of the calpain-caspase pathway but also the activation of the ER stress-induced UPR-mediated apoptosis pathways (Fig. 2). *In vitro* study results should be verified by *in vivo* studies to understand the underlying mechanism in cadmium-induced renal toxicity. Unfortunately, however, there is no evidence concerning the apoptotic cell death of kidney proximal tubular cells that is mediated by the ceramide-calpain pathway *in vivo*.

One of the causative mechanisms of nephrotoxicity caused

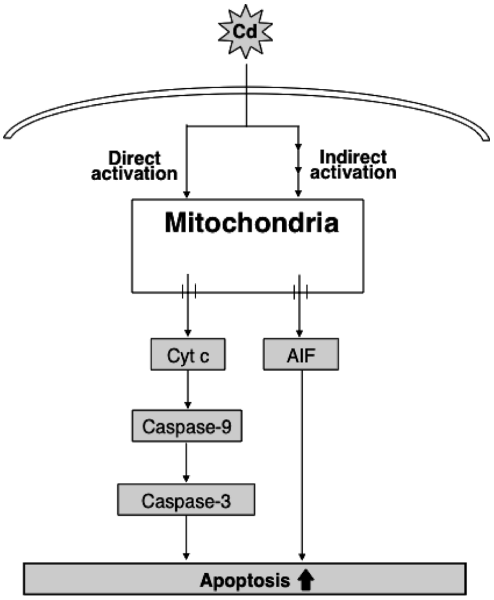

Fig. 3. Model for Cadmium-Induced Apoptosis *via* the Mitochondria-Mediated Pathway in the Kidney

Cadmium directly affects the mitochondria and consequently induces the release of mitochondria-dependent proapoptotic regulator Cyt *c*. Cyt *c* activates caspase-9 and consequently activates caspase-3. Cadmium also indirectly induces both the caspase-dependent and -independent pathways *via* the release of Cyt *c* and AIF, respectively.

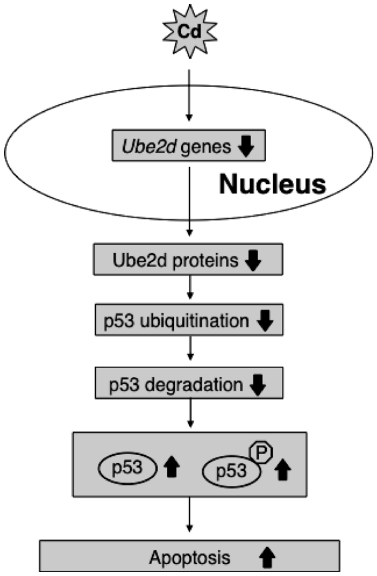

Fig. 4. Model for Cadmium-Induced Apoptosis *via* the p53-Dependent Pathway in the Kidney

Cadmium causes down-regulation of the *Ube2d* gene family and their proteins, and consequently inhibits p53 ubiquitination and its degradation that initiates p53 overaccumulation and its phosphorylation in the cells, which induces p53 DNA binding and the p53-dependent apoptotic pathway.

by chronic cadmium exposure has been suggested to be oxidative stress, whereas the molecular mechanisms for cadmium-induced oxidative stress in the kidney are not well understood.[42] In porcine kidney LLC-PK_1 cells, cadmium induces reactive oxygen species (ROS) generation; both antioxidants and stable overexpression of superoxide dismutase attenuate cadmium-induced ER stress, which activates proapoptotic UPR signaling cascades and apoptosis.[30] Although it is unclear how ROS induces ER stress in the kidney, ROS generation caused by cadmium may at least partly contribute to cadmium-induced kidney apoptosis through ER stress.

3. MITOCHONDRIA-MEDIATED PATHWAY

Caspases play a critical role in the initiation and the execution of apoptosis, and caspases are activated by either an intrinsic (mitochondria-mediated) pathway or by extrinsic (death receptor-mediated) pathways. The mitochondria-mediated intrinsic pathway is initiated by multiple stimuli, including ROS and cytosolic calcium, and the mitochondria releases proapoptotic factor cytochrome *c* (Cyt *c*), followed by the activation of caspase-9 and -3 to induce apoptosis. In kidney proximal tubule cells, cadmium can induce both caspase-9 and -3 activity, which suggests that mitochondria- and caspase-mediated apoptosis pathways are also related to cadmium-induced renal toxicity.[43] In contrast, in rat proximal tubule WKPT-0293 Cl. 2 cells and mouse renal mesangial cells, cadmium can stimulate the release of proapoptotic factors, *i.e.*, endonuclease apoptosis-inducing factor (AIF) from the mitochondria, which indicates that cadmium activates not only a caspase-dependent pathway but also caspase-independent pathways against mitochondria.[43,44] Moreover, using the mitochondria isolated from the kidney cell, cadmium has been shown to have direct effects against mitochondria because cadmium induces mitochondria swelling and subsequent Cyt *c* release.[45,46] Possible pathways for cadmium-induced mitochondria-mediated apoptosis are illustrated in Fig. 3.

4. p53-DEPENDENT PATHWAY

To determine the molecular targets and the molecular mechanisms of cadmium-induced renal toxicity, we recently examined the gene expression pattern of normal rat kidney epithelial cells (NRK-52E cells) after cadmium exposure by DNA microarray.[47] Cadmium was found to increase the expression of 73 genes and decreased the expression of 42 genes in NRK-52E cells before cytotoxic effects appeared. Among these genes, we focused on the Ub–proteasome system-related gene *Ube2d4* that was down-regulated by cadmium treatment because the expression level of Ubc4 (Ube2d family homolog) affects sensitivity to cadmium cytotoxicity in yeast cells.[48,49] The Ub–proteasome system is an ATP-dependent degradation unit for damaged or short-lived proteins.[50] Unnecessary proteins are ubiquitinated by Ub-activation enzyme (E1), Ub-conjugating enzyme (E2), Ub-ligase (E3), and Ub-chain elongation factor (E4), and subsequently, these ubiquitinated proteins are degraded by the proteasome. *Ube2d4*, which encodes Ub-conjugating enzyme E2D (Ube2d) 4, is a member of the Ube2d family. We also found that cadmium markedly and rapidly inhibited the gene expression of not only *Ube2d4* but also another *Ube2d* gene family, including *Ube2d1* (which encodes for Ube2d1), *Ube2d2* (which encodes for Ube2d2) and *Ube2d3* (which encodes for Ube2d3) in NRK-52E cells.[23] Cadmium may inhibit the degradation of Ube2d family enzyme-mediated unnecessary proteins *via* strong down-regulation of the *Ube2d* gene family non-selectively and may induce the intracellular accumulation of these unnecessary proteins in the cells. In human breast carcinoma MCF7 cells, Ube2d2 and Ube2d3 have been shown to be related to the ubiquitination

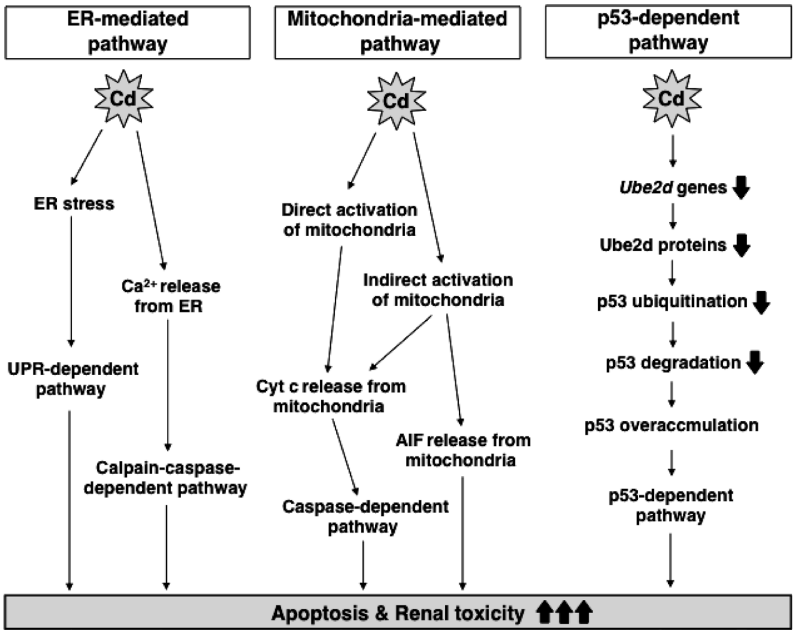

Fig. 5. Model for Three Cadmium-Induced Apoptosis Pathways in the Kidney

Cadmium induces three major apoptotic pathways in the kidney cells as follows: 1) the ER-mediated pathway *via* ER stress and calcium release that is followed by the activation of UPR-dependent and calpain-caspase-dependent apoptotic pathways, respectively; 2) the mitochondria-mediated pathway *via* direct and indirect activation of mitochondria by cadmium, followed by caspase-dependent and/or -independent pathways; and 3) the p53-dependent apoptotic pathway *via* suppression of the *Ube2d* gene family expression and p53 overaccumulation.

and degradation of tumor suppressor protein p53.[51] p53 is well known to be ubiquitinated and degraded immediately by the Ub–proteasome system,[52–55] and the amount of p53 is tightly regulated by the rate of degradation, rather than the rate of production.[56] Our recent study in NRK-52E cells demonstrated that cadmium markedly increased p53 intracellular accumulation accompanied by p53 phosphorylation and neither up-regulated the *p53* gene nor directly inhibited proteasome activity prior to the appearance of apoptotic cell death.[23] These observations suggest that cadmium-induced p53 overaccumulation is caused by the inhibition of p53 degradation in the Ub–proteasome system *via* the down-regulation of the *Ube2d* gene family, and cadmium thus induces p53-dependent apoptosis in cells.[23] The possible mechanism of cadmium-induced p53-dependent apoptosis in the kidney is illustrated in Fig. 4. Moreover, we have investigated whether these *in vitro* data obtained in the NRK-52E cell model are reflected in the kidney of mice exposed to chronic cadmium *in vivo*. Cadmium was found to inhibit the expression of all *Ube2d* genes (*Ube2d1*, *Ube2d2* and *Ube2d3*) in the kidney of mice that were chronically exposed to cadmium for 12 months, which caused mild renal toxicity. In addition, increases of p53 protein levels were also observed in the kidney, whereas *p53* mRNA levels were unchanged by cadmium. Moreover, apoptotic cell damage in the renal tubule cells, but not in glomeruli, were observed in mice that were exposed to cadmium for 12 months. Together, these results suggest that chronic cadmium exposure induces p53 overaccumulation through suppression of the *Ube2d* gene family expression in the renal tubular cells of mice, as well as NRK-52E cells, and that cadmium thus induces p53-dependent renal tubule cell apoptosis.[23] This p53-mediated apoptosis pathway caused by cadmium is supported by recent studies using small interference RNA (siRNA) of p53 as follows: p53 siRNA transfection suppresses cadmium-induced apoptosis in mouse epidermal cells[57] and human prostate epithelial cells.[19] Moreover, cadmium causes an increase in intracellular p53 protein levels and/or phosphorylated p53 protein levels in several cells.[57–65] These observations also support our findings that the p53-mediated pathway is one of the critical pathways for inducing apoptosis in chronic cadmium-exposed proximal tubular cells in the kidney.

5. CONCLUSION

In this review, we focused on cadmium-induced apoptotic pathways in the kidney and in cultured kidney cells, which are the main targeted tissues and cells after chronic cadmium exposure. Cadmium-induced apoptosis signaling pathways were separated into three pathways: 1) the ER-mediated pathway *via* ER stress and calcium release that is followed by the activation of UPR-dependent and calpain-caspase-dependent apoptotic pathways, respectively; 2) the mitochondria-mediated pathway *via* direct and indirect activation of the mitochondria by cadmium that is followed by caspase-dependent and/or -independent pathways; and 3) the p53-dependent apoptotic pathway *via* suppression of the *Ube2d* gene family expression and p53 overaccumulation (Fig. 5). Although other apoptotic pathways and factors may be involved, these three pathways are considered to play critical roles in cadmium-induced kidney cell apoptosis. However, the primary target molecules that directly interact with cadmium and the contribution ratio of each pathway have not yet been sufficiently elucidated. Moreover, there is little known about the apoptotic effects and mechanisms of cadmium in human kidney cells. However, a more recent report has shown that in HK-2 human renal proximal tubular cells, salubrinal, an ER stress inhibitor, protects against cadmium-induced apoptosis *via* the suppression of

cell death signal transduction pathways.[66] Additional studies that include human cells and animal models should elucidate the exact contribution ratio of each apoptotic pathway and determine the key therapeutic target molecules against cadmium-induced renal toxicity.

REFERENCES

1) Satoh M, Koyama H, Kaji T, Kito H, Tohyama C. Perspectives on cadmium toxicity research. *Tohoku J. Exp. Med.*, **196**, 23–32 (2002).
2) Gonick HC. Nephrotoxicity of cadmium & lead. *Indian J. Med. Res.*, **128**, 335–352 (2008).
3) Nordberg GF. Historical perspectives on cadmium toxicology. *Toxicol. Appl. Pharmacol.*, **238**, 192–200 (2009).
4) Dorian C, Gattone VH 2nd, Klaasen CD. Renal cadmium deposition and injury as a result of accumulation of cadmium-metallothionein (CdMT) by the proximal convoluted tubules—A light microscopic autoradiography study with 109CdMT. *Toxicol. Appl. Pharmacol.*, **114**, 173–181 (1992).
5) Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. *Cell*, **88**, 347–354 (1997).
6) Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.*, **16**, 663–669 (2004).
7) Fadeel B, Orrenius S, Zhivotovsky B. Apoptosis in human disease: a new skin for the old ceremony? *Biochem. Biophys. Res. Commun.*, **266**, 699–717 (1999).
8) Habeebu SS, Liu J, Klaassen CD. Cadmium-induced apoptosis in mouse liver. *Toxicol. Appl. Pharmacol.*, **149**, 203–209 (1998).
9) Ishido M, Homma-Takeda S, Tohyama C, Suzuki T; Masami Ishido Shino Homma-Takeda Ch. Apoptosis in rat renal proximal tubular cells induced by cadmium. *J. Toxicol. Environ. Health A*, **55**, 1–12 (1998).
10) Xu G, Zhou G, Jin T, Zhou T, Hammarström S, Bergh A, Nordberg G. Apoptosis and p53 gene expression in male reproductive tissues of cadmium exposed rats. *Biometals*, **12**, 131–139 (1999).
11) Zhou T, Zhou G, Song W, Eguchi N, Lu W, Lundin E, Jin T, Nordberg G. Cadmium-induced apoptosis and changes in expression of p53, c-jun and MT-I genes in testes and ventral prostate of rats. *Toxicology*, **142**, 1–13 (1999).
12) Krichah R, Ben Rhouma K, Hallègue D, Tébourbi O, Joulin V, Couton D, Sakly M. Acute cadmium administration induces apoptosis in rat thymus and testicle, but not liver. *Pol. J. Environ. Stud.*, **12**, 589–594 (2003).
13) Prozialeck WC, Edwards JR, Lamar PC, Liu J, Vaidya VS, Bonventre JV. Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of Cd-induced proximal tubule injury. *Toxicol. Appl. Pharmacol.*, **238**, 306–314 (2009).
14) el Azzouzi B, Tsangaris GT, Pellegrini O, Manuel Y, Benveniste J, Thomas Y. Cadmium induces apoptosis in a human T cell line. *Toxicology*, **88**, 127–139 (1994).
15) Li M, Kondo T, Zhao QL, Li FJ, Tanabe K, Arai Y, Zhou ZC, Kasuya M. Apoptosis induced by cadmium in human lymphoma U937 cells through Ca^{2+}-calpain and caspase-mitochondria-dependent pathways. *J. Biol. Chem.*, **275**, 39702–39709 (2000).
16) Lasfer M, Vadrot N, Aoudjehane L, Conti F, Bringuier AF, Feldmann G, Reyl-Desmars F. Cadmium induces mitochondria-dependent apoptosis of normal human hepatocytes. *Cell Biol. Toxicol.*, **24**, 55–62 (2008).
17) Ye JL, Mao WP, Wu AL, Zhang NN, Zhang C, Yu YJ, Zhou L, Wei CJ. Cadmium-induced apoptosis in human normal liver L-02 cells by acting on mitochondria and regulating Ca^{2+} signals. *Environ. Toxicol. Pharmacol.*, **24**, 45–54 (2007).
18) Shih CM, Wu JS, Ko WC, Wang LF, Wei YH, Liang HF, Chen YC, Chen CT. Mitochondria-mediated caspase-independent apoptosis induced by cadmium in normal human lung cells. *J. Cell. Biochem.*, **89**, 335–347 (2003).
19) Aimola P, Carmignani M, Volpe AR, Di Benedetto A, Claudio L, Waalkes MP, van Bokhoven A, Tokar EJ, Claudio PP. Cadmium induces p53-dependent apoptosis in human prostate epithelial cells. *PLoS ONE*, **7**, e33647 (2012).
20) Liu Y, Templeton DM. Initiation of caspase-independent death in mouse mesangial cells by Cd^{2+}: involvement of p38 kinase and CaMK-II. *J. Cell. Physiol.*, **217**, 307–318 (2008).
21) Hart BA, Lee CH, Shukla GS, Shukla A, Osier M, Eneman JD, Chiu JF. Characterization of cadmium-induced apoptosis in rat lung epithelial cells: evidence for the participation of oxidant stress. *Toxicology*, **133**, 43–58 (1999).
22) López E, Figueroa S, Oset-Gasque MJ, González MP. Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture. *Br. J. Pharmacol.*, **138**, 901–911 (2003).
23) Tokumoto M, Fujiwara Y, Shimada A, Hasegawa T, Seko Y, Nagase H, Satoh M. Cadmium toxicity is caused by accumulation of p53 through the down-regulation of Ube2d family genes *in vitro* and *in vivo*. *J. Toxicol. Sci.*, **36**, 191–200 (2011).
24) Ishido M, Tohyama C, Suzuki T. c-myc is not involved in cadmium-elicited apoptotic pathway in porcine kidney LLC-PK_1 cells. *Life Sci.*, **63**, 1195–1204 (1998).
25) Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.*, **8**, 519–529 (2007).
26) Kitamura M. Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces. *Am. J. Physiol. Renal Physiol.*, **295**, F323–F334 (2008).
27) Lee AS. The glucose-regulated proteins: stress induction and clinical applications. *Trends Biochem. Sci.*, **26**, 504–510 (2001).
28) Kaufman RJ. Orchestrating the unfolded protein response in health and disease. *J. Clin. Invest.*, **110**, 1389–1398 (2002).
29) Yokouchi M, Hiramatsu N, Hayakawa K, Kasai A, Takano Y, Yao J, Kitamura M. Atypical, bidirectional regulation of cadmium-induced apoptosis *via* distinct signaling of unfolded protein response. *Cell Death Differ.*, **14**, 1467–1474 (2007).
30) Yokouchi M, Hiramatsu N, Hayakawa K, Okamura M, Du S, Kasai A, Takano Y, Shitamura A, Shimada T, Yao J, Kitamura M. Involvement of selective reactive oxygen species upstream of proapoptotic branches of unfolded protein response. *J. Biol. Chem.*, **283**, 4252–4260 (2008).
31) Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta*, **1802**, 396–405 (2010).
32) Thévenod F. Cadmium and cellular signaling cascades: to be or not to be? *Toxicol. Appl. Pharmacol.*, **238**, 221–239 (2009).
33) Yeh JH, Huang CC, Yeh MY, Wang JS, Lee JK, Jan CR. Cadmium-induced cytosolic Ca^{2+} elevation and subsequent apoptosis in renal tubular cells. *Basic Clin. Pharmacol. Toxicol.*, **104**, 345–351 (2009).
34) Foskett JK, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol. Rev.*, **87**, 593–658 (2007).
35) Lawal AO, Ellis EM. Phospholipase C mediates cadmium-dependent apoptosis in HEK 293 cells. *Basic Clin. Pharmacol. Toxicol.*, **110**, 510–517 (2012).
36) Faurskov B, Bjerregaard HF. Evidence for cadmium mobilization of intracellular calcium through a divalent cation receptor in renal distal epithelial A6 cells. *Pflugers Arch.*, **445**, 40–50 (2002).
37) Saddoughi SA, Song P, Ogretmen B. Roles of bioactive sphingolipids in cancer biology and therapeutics. *Subcell. Biochem.*, **49**, 413–440 (2008).
38) Lee WK, Thévenod F. Novel roles for ceramides, calpains and caspases in kidney proximal tubule cell apoptosis: lessons from *in vitro* cadmium toxicity studies. *Biochem. Pharmacol.*, **76**, 1323–1332 (2008).
39) Lee WK, Torchalski B, Thévenod F. Cadmium-induced ceramide formation triggers calpain-dependent apoptosis in cultured kidney

proximal tubule cells. *Am. J. Physiol. Cell Physiol.*, **293**, C839–C847 (2007).
40) Lee WK, Torchalski B, Kohistani N, Thévenod F. ABCB1 protects kidney proximal tubule cells against cadmium-induced apoptosis: roles of cadmium and ceramide transport. *Toxicol. Sci.*, **121**, 343–356 (2011).
41) Viner RI, Hühmer AF, Bigelow DJ, Schöneich C. The oxidative inactivation of sarcoplasmic reticulum Ca^{2+}-ATPase by peroxynitrite. *Free Radic. Res.*, **24**, 243–259 (1996).
42) Gobe G, Crane D. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. *Toxicol. Lett.*, **198**, 49–55 (2010).
43) Liu Y, Templeton DM. Initiation of caspase-independent death in mouse mesangial cells by Cd^{2+}: involvement of p38 kinase and CaMK-II. *J. Cell. Physiol.*, **217**, 307–318 (2008).
44) Lee WK, Abouhamed M, Thévenod F. Caspase-dependent and -independent pathways for cadmium-induced apoptosis in cultured kidney proximal tubule cells. *Am. J. Physiol. Renal Physiol.*, **291**, F823–F832 (2006).
45) Lee WK, Bork U, Gholamrezaei F, Thévenod F. Cd^{2+}-induced cytochrome *c* release in apoptotic proximal tubule cells: role of mitochondrial permeability transition pore and Ca^{2+} uniporter. *Am. J. Physiol. Renal Physiol.*, **288**, F27–F39 (2005).
46) Lee WK, Spielmann M, Bork U, Thévenod F. Cd^{2+}-induced swelling-contraction dynamics in isolated kidney cortex mitochondria: role of Ca^{2+} uniporter, K^+ cycling, and protonmotive force. *Am. J. Physiol. Cell Physiol.*, **289**, C656–C664 (2005).
47) Tokumoto M, Ohtsu T, Honda A, Fujiwara Y, Nagase H, Satoh M. DNA microarray analysis of normal rat kidney epithelial cells treated with cadmium. *J. Toxicol. Sci.*, **36**, 127–129 (2011).
48) Jungmann J, Reins HA, Schobert C, Jentsch S. Resistance to cadmium mediated by ubiquitin-dependent proteolysis. *Nature*, **361**, 369–371 (1993).
49) Hwang GW, Furuchi T, Naganuma A. The ubiquitin-conjugating enzymes, Ubc4 and Cdc34, mediate cadmium resistance in budding yeast through different mechanisms. *Life Sci.*, **82**, 1182–1185 (2008).
50) Ciehanover A, Hod Y, Hershko A. A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem. Biophys. Res. Commun.*, **81**, 1100–1105 (1978).
51) Saville MK, Sparks A, Xirodimas DP, Wardrop J, Stevenson LF, Bourdon JC, Woods YL, Lane DP. Regulation of p53 by the ubiquitin-conjugating enzymes UbcH5B/C *in vivo*. *J. Biol. Chem.*, **279**, 42169–42181 (2004).
52) Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature*, **387**, 296–299 (1997).
53) Vucic D, Dixit VM, Wertz IE. Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. *Nat. Rev. Mol. Cell Biol.*, **12**, 439–452 (2011).
54) Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.*, **420**, 25–27 (1997).
55) Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature*, **387**, 299–303 (1997).
56) Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*, **408**, 307–310 (2000).
57) Son YO, Lee JC, Hitron JA, Pan J, Zhang Z, Shi X. Cadmium induces intracellular Ca^{2+}- and H_2O_2-dependent apoptosis through JNK- and p53-mediated pathways in skin epidermal cell line. *Toxicol. Sci.*, **113**, 127–137 (2010).
58) Figueiredo-Pereira ME, Li Z, Jansen M, Rockwell P. *N*-Acetylcysteine and celecoxib lessen cadmium cytotoxicity which is associated with cyclooxygenase-2 up-regulation in mouse neuronal cells. *J. Biol. Chem.*, **277**, 25283–25289 (2002).
59) Yu X, Hong S, Faustman EM. Cadmium-induced activation of stress signaling pathways, disruption of ubiquitin-dependent protein degradation and apoptosis in primary rat Sertoli cell-gonocyte cocultures. *Toxicol. Sci.*, **104**, 385–396 (2008).
60) Yu X, Robinson JF, Sidhu JS, Hong S, Faustman EM. A system-based comparison of gene expression reveals alterations in oxidative stress, disruption of ubiquitin–proteasome system and altered cell cycle regulation after exposure to cadmium and methylmercury in mouse embryonic fibroblast. *Toxicol. Sci.*, **114**, 356–377 (2010).
61) Kim BJ, Kim MS, Kim KB, Kim KW, Hong YM, Kim IK, Lee HW, Jung YK. Sensitizing effects of cadmium on TNF-α- and TRAIL-mediated apoptosis of NIH3T3 cells with distinct expression patterns of p53. *Carcinogenesis*, **23**, 1411–1417 (2002).
62) Lag M, Westly S, Lerstad T, Bjørnsrud C, Refsnes M, Schwarze PE. Cadmium-induced apoptosis of primary epithelial lung cells: involvement of Bax and p53, but not of oxidative stress. *Cell Biol. Toxicol.*, **18**, 29–42 (2002).
63) Dai W, Chen H, Yu R, He L, Chen B, Chen X. Effects of cadmium on telomerase activity, expressions of TERT, c-myc and P53, and apoptosis of rat hepatocytes. *J. Huazhong Univ. Sci. Technolog. Med. Sci.*, **30**, 709–713 (2010).
64) Matsuoka M, Igisu H. Cadmium induces phosphorylation of p53 at serine 15 in MCF-7 cells. *Biochem. Biophys. Res. Commun.*, **282**, 1120–1125 (2001).
65) Achanzar WE, Achanzar KB, Lewis JG, Webber MM, Waalkes MP. Cadmium induces c-myc, p53, and c-jun expression in normal human prostate epithelial cells as a prelude to apoptosis. *Toxicol. Appl. Pharmacol.*, **164**, 291–300 (2000).
66) Komoike Y, Inamura H, Matsuoka M. Effects of salubrinal on cadmium-induced apoptosis in HK-2 human renal proximal tubular cells. *Arch. Toxicol.*, **86**, 37–44 (2012).