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The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity

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Abstract Cadmium preferentially accumulates in the kidney, the major target for cadmium-related toxicity. Several underlying mechanisms are postulated, and reactive oxygen species (ROS) have been considered as crucial mediators for tissue injuries. In addition to oxidative stress, we recently disclosed that endoplasmic reticulum (ER) stress also plays a critical role. Cadmium causes ER stress in vitro and in vivo and mediates induction of apoptosis in target tissues. In this article, we describe a role for ER stress and involvement of particular branches of the unfolded protein response (UPR) in cadmium-triggered tissue injury, especially nephrotoxicity. We also discuss relationship between oxidative stress and ER stress, and involvement of selective ROS in the induction of pro-apoptotic branches of the UPR.

Keywords Cadmium · Endoplasmic reticulum stress · Unfolded protein response · Reactive oxygen species · Apoptosis · Nephrotoxicity

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Introduction

Cadmium is a toxic metal and a potent environmental pollutant. In humans, cadmium intoxication is caused by its intake through contaminated water, food and air, especially cigarette smoke. Because of its low excretion rate, cadmium accumulates in different organs over time and causes a wide range of negative effects on human's health, including renal dysfunction, osteoporosis/bone fractures and development of cancers (Järup and Åkesson 2009). Several underlying mechanisms have been postulated, and currently, reactive oxygen species (ROS) are considered as crucial mediators for the cadmium-triggered tissue injuries (Liu et al. 2009). However, recent investigation disclosed that, in addition to oxidative stress, cadmium causes endoplasmic reticulum (ER) stress in vitro and in vivo, which also plays a critical role in the induction of apoptosis by cadmium (Yokouchi et al. 2007; Yokouchi et al. 2008). In this article, we describe a role of ER stress and the consequent unfolded protein response (UPR) in cadmium-triggered cell damage, especially focusing on renal cell injury.

Cadmium-triggered renal injury

Exposure to cadmium via drinking water, foods and cigarette smoke causes accumulation of this metal in some target organs, especially in the kidney and liver (Lind et al. 1997). In particular, the highest

concentrations are observed in the kidney, which is considered as the major target for the cadmium-related toxicity (Nogawa 1981; Hamada et al. 1991). Human studies indicate that 7% of the general population have renal dysfunction caused by cadmium exposure (Friberg et al. 1986). Cadmium nephropathy is characterized by proteinuria, aminoaciduria, glucosuria, phosphaturia and reduction in glomerular filtration rate (Nogawa 1981). In the kidney, accumulation of cadmium occurs mainly in the proximal tubules, and other nephron segments are affected only at later stages of the intoxication (Bernard 2004). Following prolonged and/or high levels of exposure to cadmium, apoptosis is induced in the proximal tubules (Ishido et al. 1998). The tubular injury progresses to glomerular damage with reduction in glomerular filtration rate, leading to renal dysfunction. The World Health Organization defines that the critical cadmium concentration in the renal cortex is 200 mg/kg cortex wet weight. Above this threshold, renal dysfunction is caused (WHO 1992).

Previous investigations suggest that toxic effects of cadmium on the proximal tubules are caused through several mechanisms (Thevenod 2003). In vitro, longer-term exposures of tubular cells to cadmium result in a decrease in glutathione levels and consequent cellular death (Prozialeck and Lamar 1995), indicating involvement of oxidative stress. Cytosolic cadmium generates ROS which deplete endogenous radical scavengers. ROS also damage a variety of transport proteins including Na^+/K^+-ATPase, resulting in their degradation by the proteasome and endo-lysosomal pathways (Thevenod 2003). It causes tubular cell dysfunction.

Roles of oxidative stress in cadmium-induced cell death

ROS have been implicated in cadmium toxicity, using either a variety of cell culture systems or animal models. Cadmium depletes glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of ROS such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (Liu et al. 2009). The spin-trapping technique in conjunction with electron spin resonance also provided direct evidence for cadmium-triggered generation of these ROS in vitro and in vivo (Liu et al. 2008).

The sequential reduction of oxygen leads to generation of $O_2^{\cdot-}$ and H_2O_2 (Jacobson 1996). $O_2^{\cdot-}$ also rapidly reacts with nitric oxide (NO), yielding another reactive species peroxynitrite ($ONOO^-$) (Szabo and Ohshima 1997). All of these ROS have the potential to trigger cellular death (Jacobson 1996; Szabo and Ohshima 1997; Buttke and Sandstromb 1994). Directly, ROS oxidize proteins, lipids and nucleic acids, leading to damage of the basic cell structures. ROS also oxidize the catalytic cysteine in the enzymes involved in apoptotic and necrotic signaling pathways. For example, oxidative stress activates the ASK1 (apoptosis signal-regulating kinase 1) - JNK (c-Jun N-terminal kinase) pathway implicated in a variety of apoptotic processes (Pourova et al. 2010). However, molecular mechanisms involved in the induction of the pro-apoptotic pathways by ROS are not fully elucidated. Accumulating evidence suggests that protein folding and generation of ROS (as a byproduct of protein oxidation in the ER) are closely linked with each other (Malhotra and Kaufman 2007). Several recent reports indicate that ER stress locates downstream of oxidative stress in some apoptotic processes triggered by tumor necrosis factor-α (TNF-α), cigarette smoke, and cadmium (Xue et al. 2005; Tagawa et al. 2008; Yokouchi et al. 2008).

ER stress and UPR

ER stress is defined as accumulation of unfolded or misfolded proteins in the ER. It induces a coordinated adaptive program, namely the UPR. Three major transducers for sensing ER stress have been identified on the membrane of the ER; i.e., RNA-dependent protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1). Activation of PERK leads to phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which causes general inhibition of protein synthesis. In response to ER stress, 90 kDa ATF6 (p90ATF6) transits to the Golgi where it is cleaved by site-1 protease and site-2 protease, yielding an active transcription factor, 50 kDa ATF6 (p50ATF6). Similarly, activated IRE1 catalyzes removal of a small intron from the mRNA of X-box binding protein 1 (XBP1). This splicing event creates a translational frameshift in

XBP1 mRNA to produce an active transcription factor. Active p50ATF6 and XBP1 subsequently bind to the ER stress response element (ERSE) and the UPR element (UPRE), leading to expression of target genes including ER chaperone 78 kDa glucose-regulated protein (GRP78) (also called Bip) and ER-associated degradation (ERAD) factors involved in degradation of unfolded proteins (Wu and Kaufman 2006; Kitamura 2008) (Fig. 1). Primarily, these pathways contribute to attenuation of ER stress.

Induction of apoptosis by ER stress and consequent UPR

During the UPR, however, death signals may also be transduced (Kim et al. 2006). For example, activation of the PERK—eIF2α pathway causes selective induction of transcription factor ATF4. Subsequently, ATF4 induces expression of pro-apoptotic CCAAT/enhancer-binding protein-homologous protein (CHOP) [also called growth arrest and DNA damage-inducible protein 153 (GADD153)] through binding to the amino acid response element (AARE) (Harding et al. 2000). The regulatory region of the CHOP gene contains ERSEs, and the ATF6 pathway and the IRE1 pathway may also induce expression of CHOP via activation of the ERSE (Gotoh et al. 2002). ER stress activates caspase-12 localized at the ER membrane through an interaction with IRE1 and TNF receptor-associated factor 2 (TRAF2), leading cells to undergo apoptosis. The IRE1—TRAF2 interaction also allows for recruitment and activation of ASK1 and downstream JNK, both of which are involved in a variety of pro-apoptotic signaling (Kim et al. 2006) (Fig. 2).

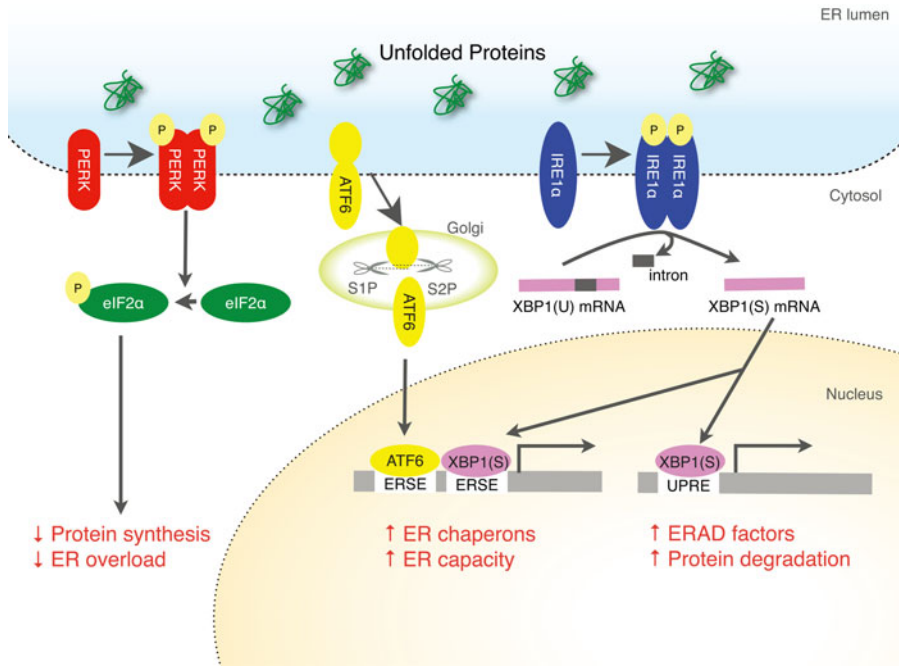

Fig. 1 ER stress and UPR. Accumulation of unfolded proteins in the ER causes ER stress, leading to activation of three major branches of the UPR. Dimerization and phosphorylation of PERK leads to phosphorylation of eIF2α that causes general inhibition of protein synthesis. In response to ER stress, 90 kDa ATF6 transits to the Golgi where it is cleaved by site-1 protease (S1P) and site-2 protease (S2P), yielding an active transcription factor, 50 kDa ATF6. Similarly, dimerization and phosphorylation of IRE1 catalyzes removal of a small intron from XBP1 mRNA [XBP1(U)]. This splicing event creates a translational frameshift in *XBP1* mRNA to produce an active transcription factor XBP1(S). p50ATF6 and XBP1(S) subsequently bind to the ERSE and UPRE, leading to expression of target genes including ER chaperones and ERAD factors. These pathways attenuate ER stress by reducing ER overload, increasing ER capacity, and enhancing degradation of unfolded proteins

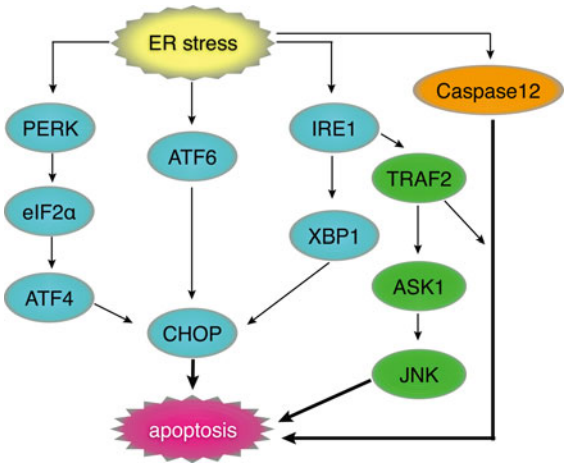

Fig. 2 Induction of apoptosis by ER stress via UPR. Activation of the PERK—eIF2α pathway causes selective induction of ATF4, leading to induction of pro-apoptotic CHOP through binding to the amino acid response element. The ATF6 pathway and the IRE1—XBP1 pathway may also induce expression of CHOP via activation of the ERSE. ER stress activates caspase-12 through an interaction with IRE1 and TRAF2, leading cells to undergo apoptosis. The IRE1—TRAF2 interaction also allows for recruitment and activation of ASK1 and downstream JNK, leading to apoptosis

Evidence for induction of ER stress by cadmium

Previous reports suggest that some heavy metals, including manganese, lead and mercury, may induce ER stress and contribute to cellular damage in the brain (Chun et al. 2001; Qian et al. 2001). Similarly, cadmium also has the potential to induce ER stress in various cell types. For example, cadmium triggers expression of GRP94 in thymocytes and GRP78 in lung epithelial cells and renal tubular cells (Kwon et al. 1999; Timblin et al. 1998; Liu et al. 2006). Cadmium causes expression of CHOP, activation of caspase-12 and consequent apoptosis in mesangial cells and hepatoma cells (Wang et al. 2009; Tchounwou et al. 2001). It also induces splicing of XBP1 mRNA and activation of caspase-12 in fibroblasts (Biagioli et al. 2008). Cadmium triggers the PERK—eIF2α—ATF4 pathway, and it is more prominent compared with its induction by other heavy metals (Liu et al. 2006). We showed that, in renal tubular cells, cadmium induces expression of GRP78, GRP94 and CHOP. Administration of cadmium in mice also triggers expression of GRP78 and CHOP in the kidney (Yokouchi et al. 2007). In response to cadmium, phosphorylation of PERK and eIF2α, activation of ATF6 and splicing of XBP1 mRNA are observed in renal tubular cells (Yokouchi et al. 2007), suggesting that cadmium can activate three major branches of the UPR.

We previously reported that ER stress-responsive alkaline phosphatase (ES-TRAP) is a sensitive, quantitative marker for ER stress (Hiramatsu et al. 2006). In vitro, activity of ES-TRAP secreted by transfected cells is rapidly down-regulated in response to ER stress independent of transcriptional regulation. The decrease in ES-TRAP activity is caused by abnormal post-translational modification, accelerated degradation and reduced secretion of ES-TRAP protein. This phenomenon is observed in a wide range of cell types triggered by various ER stress inducers (Hiramatsu et al. 2006). Using this reporter system, we generated transgenic sensor mice systemically expressing ES-TRAP and found that in vivo monitoring of ER stress is feasible by measuring the level of serum ES-TRAP activity within 2 h (Hiramatsu et al. 2006). When ES-TRAP mice are administered with cadmium intraperitoneally, activity of serum ES-TRAP is rapidly down-regulated (Hiramatsu et al. 2007). Consistent with this result, GRP78 is markedly induced in the liver and kidney following administration of cadmium. Interestingly, induction of GRP78 is not evident in other organs including the brain, heart, lung and spleen (Hiramatsu et al. 2007). These results provide evidence for the potential of cadmium to induce ER stress.

Involvement of ER stress and particular branches of the UPR in cadmium-induced apoptosis

Cadmium induces both ER stress and apoptosis in vitro and in vivo. However, information is limited regarding whether apoptosis caused by cadmium is ascribed to induction of ER stress. To examine this possibility, renal tubular cells were stably transfected with the ER chaperone GRP78, and ER stress-resistant cells were established. We found that the GRP78-transfected cells are resistant to cadmium-induced apoptosis, as well as to tunicamycin-induced apoptosis. Consistent with this result, overexpression of 150 kDa oxygen-regulated protein (ORP150), another ER chaperone that protects cells from ER stress, similarly inhibited cadmium-induced apoptosis of tubular cells (Yokouchi et al. 2007). These results

suggest that cadmium induces apoptosis through induction of ER stress.

As described above, there are three proximal transmembrane proteins in the ER for sensing of ER stress. The first transducer is PERK, the activation of which leads to phosphorylation of eIF2α and blockade of protein synthesis. We found that cadmium rapidly induced phosphorylation of PERK and downstream eIF2α in renal tubular cells. To examine whether this signaling pathway participates in the regulation of apoptosis, salubrinal, a selective inhibitor of eIF2α dephosphorylation, was used to activate the eIF2α pathway. In tubular cells, salubrinal effectively induced phosphorylation of eIF2α and significantly inhibited cadmium-induced apoptosis (Yokouchi et al. 2007). To further confirm the anti-apoptotic role of the PERK—eIF2α pathway, cells were transiently transfected with a dominant-negative mutant of eIF2α and exposed to cadmium. In contrast to the effect of salubrinal, dominant-negative inhibition of eIF2α significantly enhanced cadmium-induced apoptosis (Yokouchi et al. 2007).

We next examined involvement of the ATF6 pathway in cadmium-induced apoptosis. We found that cadmium rapidly activated ATF6 in tubular cells. Treatment of the cells with 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), an inhibitor of ATF6, abrogated activation of ATF6 by cadmium and inhibited cadmium-induced apoptosis in a dose-dependent manner (Yokouchi et al. 2007). To further confirm the pro-apoptotic role of the ATF6 pathway, cells were transiently transfected with a dominant-negative mutant of ATF6, and the effect of cadmium on apoptosis was retested. Consistent with the effect of AEBSF, dominant-negative inhibition of ATF6 significantly suppressed cadmium-induced apoptosis (Yokouchi et al. 2007).

It is well known that ATF6 induces expression of ER chaperones via binding to ERSE (Lee 2001). However, the regulatory region of the pro-apoptotic gene CHOP also contains ERSE, and ATF6 may induce expression of CHOP in some cell types (Gotoh et al. 2002). We investigated a role of the ATF6 pathway in the induction of CHOP by cadmium. We found that inhibition of ATF6 by AEBSF blunted induction of CHOP in response to cadmium. Furthermore, transfection with a dominant-negative mutant of CHOP significantly attenuated cadmium-induced apoptosis. These results indicate that the ATF6 pathway activated by cadmium participates in the induction of apoptosis, at least in part, via induction of CHOP (Yokouchi et al. 2007).

The third proximal transducer for ER stress is IRE1. Activated IRE1 catalyzes the removal of a small intron from XBP1 mRNA, leading to production of the active transcription factor. We found that treatment with cadmium rapidly induces splicing of XBP1 mRNA in tubular cells (Yokouchi et al. 2007). Transfection with a dominant-negative mutant of XBP1 exhibited resistance against cadmium-induced apoptosis. The pro-apoptotic effect of XBP1 was independent of GRP78 and CHOP, because induction of GRP78 and CHOP by cadmium was not affected by dominant-negative inhibition of XBP1 (Yokouchi et al. 2007). Similarly to the pro-apoptotic effect of XBP1, JNK, another molecule downstream of IRE1, was also phosphorylated by cadmium, which contributed to induction of apoptosis (Yokouchi et al. 2007). Interestingly, transfection of the cells with the spliced form of XBP1 rather enhanced cadmium-triggered phosphorylation of JNK (Yokouchi et al. 2008). These results indicate the possible involvement of XBP1 in the activation of JNK by cadmium.

Taken together, these results suggest bidirectional regulation of apoptosis by the UPR in cadmium-exposed cells. The ATF6 pathway and the IRE1 pathway cooperatively cause apoptosis of tubular cells via induction of CHOP, activation of XBP1 and phosphorylation of JNK, and the PERK—eIF2α pathway counteracts the pro-apoptotic processes (Fig. 3).

Relationship between ER stress and ROS in cadmium-induced apoptosis

In addition to ER stress, oxidative stress also plays a crucial role in the induction of apoptosis in cadmium-exposed tubular cells. Previous reports showed that ROS are generated in tubular cells in response to cadmium (Prozialeck and Lamar 1995; Thevenod et al. 2000; Gennari et al. 2003; Yokouchi et al. 2008). When cells are treated with antioxidants, induction of apoptosis by cadmium is attenuated (Thevenod et al. 2000; Yokouchi et al. 2008). However, the relationship between oxidative stress and ER stress is not well understood. Haynes et al. reported that prolonged activation of the UPR

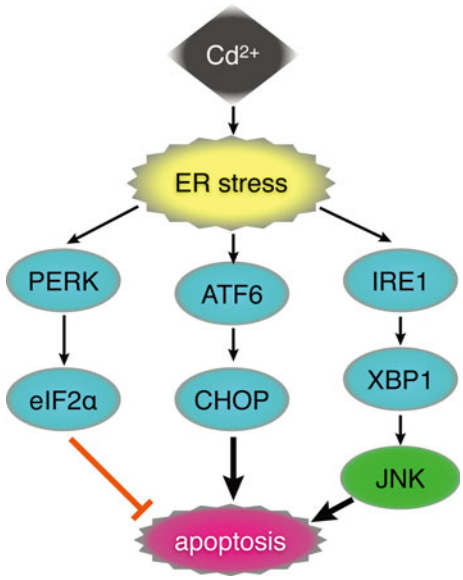

Fig. 3 Regulation of cadmium-induced apoptosis by ER stress and UPR. Cadmium induces apoptosis through induction of ER stress. Activation of the ATF6 pathway causes induction of CHOP and thereby contributes to apoptosis. Activation of the IRE1 pathway leads to induction of XBP1 and phosphorylation of JNK, both of which cooperatively contribute to apoptosis. On the other hand, the PERK—eIF2α pathway is also activated and counteracts the pro-apoptotic processes

resulted in oxidative stress and consequent cellular death. Accumulation of ROS by UPR may be caused through two mechanisms; the ER-dependent and the mitochondria-dependent ROS generation (Haynes et al. 2004). On the other hand, accumulating evidence suggests that activation of the UPR occurs on the exposure to oxidative stress, and it is an adaptive mechanism to preserve cell function and survival, as reviewed by Malhotra and Kaufman (Malhotra and Kaufman 2007). We found that cadmium-induced ER stress is attenuated by antioxidants in renal tubular cells. Exposure of the cells to ROS donors caused ER stress. In contrast, suppression of ER stress by overexpression of GRP78 or ORP150 did not attenuate cadmium-triggered oxidative stress, suggesting that ER stress is the event downstream of oxidative stress (Yokouchi et al. 2008).

The phenomenon described above may be not specific to the particular cell type and the particular stimulus. We found that exposure of bronchial epithelial cells to cigarette smoke caused ER stress and apoptosis, and attenuation of ER stress by overexpression of ER chaperones significantly suppressed the apoptotic process. Exposure of the cells to cigarette smoke caused generation of ROS, and treatment with antioxidants also inhibited cigarette smoke-induced apoptosis (Tagawa et al. 2008). Like in cadmium-exposed renal tubular cells, antioxidants blunted induction of CHOP in cigarette smoke-exposed bronchial epithelial cells, suggesting ER stress mediates apoptosis downstream of oxidative stress (Tagawa et al. 2008).

Selective involvement of $O_2^{\cdot-}$ in cadmium-triggered, UPR-dependent apoptosis

As described, ER stress is the crucial event downstream of oxidative stress in cadmium-induced apoptosis. However, information is limited which ROS play major roles in cadmium-induced ER stress and apoptosis. We found that exposure to $O_2^{\cdot-}$, H_2O_2 or $ONOO^-$ induces apoptosis of tubular cells, whereas ER stress (indicated by expression of GRP78, GRP94 and CHOP) is caused only by $O_2^{\cdot-}$ and $ONOO^-$. Transfection with manganese superoxide dismutase (MnSOD), the scavenger of $O_2^{\cdot-}$, significantly attenuated cadmium-induced ER stress and apoptosis, whereas pharmacological inhibition of $ONOO^-$ was ineffective. Interestingly, transfection with catalase attenuated cadmium-induced apoptosis partially without affecting the level of ER stress (Yokouchi et al. 2008). These results suggest; (1) cadmium causes ER stress via generation of ROS, and (2) $O_2^{\cdot-}$ is selectively involved in cadmium-triggered, ER stress-mediated apoptosis.

Cadmium activates the ATF6—CHOP pathway and the IRE1—XBP1 pathway, both of which contribute to the induction of apoptosis. We examined roles of $O_2^{\cdot-}$ in the activation of these pro-apoptotic UPR. Treatment of tubular cells with $O_2^{\cdot-}$ donor activated ATF6, and activation of ATF6 by cadmium was attenuated by transfection with MnSOD. Induction of CHOP was repressed in MnSOD-transfected cells, but not in catalase-transfected cells (Yokouchi et al. 2008). Similarly, following exposure to $O_2^{\cdot-}$, the spliced form of XBP1 mRNA was induced, and transfection with MnSOD blunted this response. $O_2^{\cdot-}$ also induced rapid phosphorylation of JNK, and it was attenuated by dominant-negative inhibition of XBP1. Furthermore,

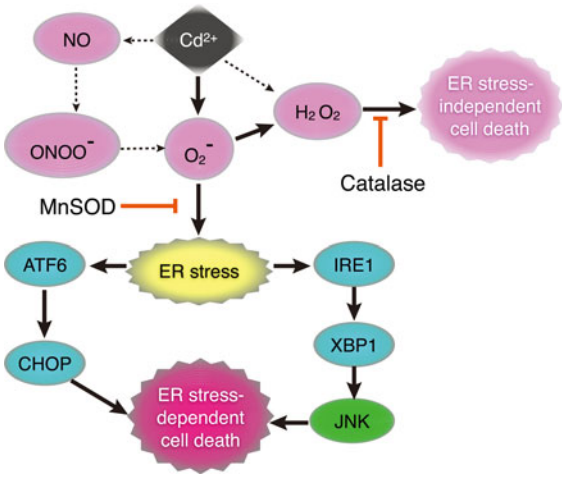

Fig. 4 Selective involvement of $O_2^{\cdot-}$ in cadmium-triggered, UPR-dependent apoptosis. Cadmium causes ER stress via generation of ROS. $O_2^{\cdot-}$ is selectively involved in cadmium-triggered, ER stress-dependent cellular death; i.e., it triggers activation of both the ATF6—CHOP and the IRE1—XBP1 pro-apoptotic pathways. $O_2^{\cdot-}$ also induces phosphorylation of JNK, which may be located downstream of XBP1. Other reactive oxygen/nitrogen species including NO, $ONOO^-$ and H_2O_2 may be generated in response to cadmium but do not contribute to cadmium-triggered, ER stress-dependent cellular death. H_2O_2 may play a role in cadmium-induced cell death in an ER stress-independent manner

transfection of the cells with the splice form of XBP1 rather enhanced $O_2^{\cdot-}$-triggered phosphorylation of JNK (Yokouchi et al. 2008). These results suggest a crucial role of $O_2^{\cdot-}$ in the activation of the ATF6—CHOP and IRE1—XBP1—JNK pro-apoptotic pathways in cadmium-exposed cells (Fig. 4).

Nuclear factor-κB (NF-κB): a downstream target for the ROS—ER stress pro-apoptotic pathway

Xie and Shaikh reported that cadmium suppresses activity of NF-κB, a well-known anti-apoptotic transcription factor, and thereby induces apoptosis of tubular epithelial cells (Xie and Shaikh 2006). They showed; (1) cadmium suppressed basal and TNF-α-inducible activity of NF-κB in a concentration-dependent manner, (2) it was associated with down-regulation of NF-κB-dependent gene products cIAP-1 and cIAP-2, (3) antioxidants preserved activity of NF-κB in cadmium-exposed cells, (4) overexpression of p65 NF-κB subunit protected the cells from cadmium-induced apoptosis, and (5) suppression of NF-κB rather potentiated apoptosis. However, molecular mechanisms involved in the suppression of NF-κB by cadmium are unknown. We disclosed that preceding ER stress blunts basal and cytokine-inducible activation of NF-κB, as reviewed recently (Kitamura 2009). This phenomenon is observed in a wide range of cell types triggered by various ER stress inducers (Takano et al. 2007; Okamura et al. 2008; Hayakawa et al. 2008; Hayakawa et al. 2009; Du et al. 2009; Harama et al. 2009; Hayakawa et al. 2010). As described in this Review, cadmium has the strong potential to induce ER stress. Possibly, cadmium suppresses NF-κB via induction of ER stress, the mechanism of which may be involved in the cadmium-triggered, ER stress-mediated apoptosis.

Several possibilities are proposed for the mechanisms underlying the suppression of NF-κB by ER stress. One possibility is induction of CCAAT/enhancer-binding protein (C/EBP), especially C/EBPβ. It is known that C/EBP family members interact with NF-κB subunits (Stein et al. 1993) and can inhibit cytokine-induced activation of NF-κB (Brasier et al. 1990; Weber et al. 2003). The inhibitory effect of C/EBP on NF-κB may be mediated by protein–protein interactions distinct from the role of C/EBP in gene expression (McKnight 2001). We found that, among C/EBP family members, C/EBPβ is preferentially induced by ER stress in several cell types including renal tubular cells (Hayakawa et al. 2009; Du et al. 2009; Hayakawa et al. 2010). Overexpression of C/EBPβ suppressed activation of NF-κB, whereas knockdown of C/EBPβ reversed the suppressive effect of ER stress. Consistent with these results, preconditioning of mice with ER stress caused induction of C/EBPβ in the kidney and suppressed NF-κB-dependent gene expression in response to lipopolysaccharide (Hayakawa et al. 2010). The down-regulation of NF-κB by ER stress may also be caused by the induction of A20, a negative feedback regulator for NF-κB. A20 is induced in several cell types (including tubular cells) under ER stress conditions (Hayakawa et al. 2009). Transfection with A20 suppressed activation of NF-κB in cytokine-stimulated cells, whereas knockdown of A20 significantly reversed the suppression of NF-κB by ER stress (Hayakawa et al. 2009). Another possibility involved in the suppression of NF-κB by ER stress is via enhanced degradation of TRAF2

(Hu et al. 2006), an essential component of the TNF signaling pathway. In our experience, the similar suppression of TRAF2 was also observed in renal mesangial cells, podocytes and tubular cells under ER stress conditions (Okamura et al. 2008; Hayakawa et al. 2009; and our unpublished observation). Taken together, these data indicate that cadmium causes ER stress and thereby inhibits activation of NF-κB through multiple mechanisms, leading to enhancement of cellular injury.

Future perspectives

In this review, we summarized a role for the oxidative stress—ER stress axis in cadmium-induced toxicity. We especially focused on the role of the oxidative stress in cadmium-triggered, ER stress-mediated apoptosis. However, other triggers may also be involved in the induction of ER stress by cadmium. Several recent reports suggest release of calcium from the ER mediates cadmium-induced apoptosis in renal tubular cells and other cell types (Xie et al. 2010; Yeh et al. 2009). Depletion of calcium store in the ER is a well-known trigger to induce ER stress. The induction of ER stress by cadmium may, in part, mediated by mobilization of intracellular calcium. Further investigation will be required to disclose the link between ER stress and the calcium signaling.

Previous reports suggested that antioxidants may be useful for prevention of cadmium-related tissue injury. In addition to antioxidants, some reagents that attenuate ER stress may also be useful for this purpose. One such strategy involves the use of chemical chaperones (Chaudhuri and Paul 2006). For example, 4-phenylbutyric acid (4-PBA) is a chemical that stabilizes protein conformation, improves ER folding capacity, and facilitates the trafficking of mutant proteins. Oral administration of 4-PBA to a murine model of type 2 diabetes alleviated ER stress, reduced diabetic symptoms, and lowered systemic inflammation (Ozcan et al. 2006). Tauroursodeoxycholic acid is another chemical chaperone that reduces ER stress and is cytoprotective and anti-apoptotic in animal models of intracerebral hemorrhage where ER stress plays a pathological role (Rodrigues et al. 2003). Based on the fact that ER stress is located downstream of oxidative stress, these chemical chaperones should be useful for prevention and treatment of cadmium-induced toxicity. In addition, several other heavy metals including manganese, lead, mercury, copper, nickel and cobalt also have the potential to induce ER stress in renal tubular cells and other cell types (Yokouchi et al. 2007; Chun et al. 2001; Qian et al. 2001). The similar mechanism described here could be involved in other metal-induced toxicity, and chemical chaperoning might be a strategy for the treatment of various heavy metal intoxication.

Accumulating evidence suggests roles of ER stress and the UPR in a wide range of pathophysiologies. In particular, cellular injury caused by ER stress has been extensively studied in various pathological contexts. Acute ER stress also causes tissue inflammation through activation of NF-κB and JNK (Pahl and Baeuerle 1995; Sekine et al. 2006). It may contribute to renal fibrogenesis observed in cadmium nephrotoxicity. However, the UPR triggered by ER stress has Janus faces. It is an adaptive mechanism that possesses the light side as well as the dark side (Kitamura 2008; Kitamura 2009). It should be noted that the UPR may also be involved in the attenuation of cell injury and inflammation during cadmium intoxication.

References

Bernard A (2004) Renal dysfunction induced by cadmium: biomarkers of critical effects. Biometals 17:519–523

Biagioli M, Pifferi S, Ragghianti M, Bucci S, Rizzuto R, Pinton P (2008) Endoplasmic reticulum stress and alteration in calcium homeostasis are involved in cadmium-induced apoptosis. Cell Calcium 43:184–195

Brasier AR, Ron D, Tate JE, Habener JF (1990) A family of constitutive C/EBP-like DNA binding proteins attenuate the IL-1α induced, NF-κB mediated trans-activation of the angiotensinogen gene acute-phase response element. EMBO J 9:3933–3944

Buttke TM, Sandstromb PA (1994) Oxidative stress as a mediator of apoptosis. Immunol Today 15:7–10

Chaudhuri TK, Paul S (2006) Protein-misfolding diseases and chaperone-based therapeutic approaches. FEBS J 273: 1331–1349

Chun HS, Lee H, Son JH (2001) Manganese induces endoplasmic reticulum (ER) stress and activates multiple caspases in nigral dopaminergic neuronal cells, SN4741. Neurosci Lett 316:5–8

Du S, Hiramatsu N, Hayakawa K, Kasai A, Okamura M, Huang T, Yao J, Takeda M, Araki I, Sawada N, Paton AW, Paton JC, Kitamura M (2009) Suppression of NF-κB by cyclosporine A and tacrolimus (FK506) via induction of the C/EBP family: implication for unfolded protein response. J Immunol 182:7201–7211

Friberg L, Elinder CG, Kjellstrom T, Nordberg GF (1986) Cadmium and health: a toxicological and epidemiological approach, vol 1, 2. CRC Press, Boca Raton

Gennari A, Cortese E, Boveri M, Casado J, Prieto P (2003) Sensitive endpoints for evaluating cadmium-induced acute toxicity in LLC-PK1 cells. Toxicology 183:211–220

Gotoh T, Oyadomari S, Mori K, Mori M (2002) Nitric oxide-induced apoptosis in RAW 264.7 macrophages is mediated by endoplasmic reticulum stress pathway involving ATF6 and CHOP. J Biol Chem 277:12343–12350

Hamada T, Nakano S, Iwai S, Tanimoto A, Ariyoshi K, Koide O (1991) Pathological study on beagles after long-term oral administration of cadmium. Toxicol Pathol 19:138–147

Harama D, Koyama K, Mukai M, Shimokawa N, Miyata M, Nakamura Y, Ohnuma Y, Ogawa H, Matsuoka S, Paton AW, Paton JC, Kitamura M, Nakao A (2009) A sub-cytotoxic dose of subtilize cytotoxin prevents LPS-induced inflammatory responses, depending on its capacity to induce the unfolded protein response. J Immunol 183:1368–1374

Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, Ron D (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 6:1099–1108

Hayakawa K, Hiramatsu N, Okamura M, Yao J, Paton AW, Paton JC, Kitamura M (2008) Blunted activation of NF-κB and NF-κB-dependent gene expression by geranylgeranylacetone: involvement of unfolded protein response. Biochem Biophys Res Commun 365:47–53

Hayakawa K, Hiramatsu N, Okamura M, Yamazaki H, Nakajima S, Yao J, Paton AW, Paton JC, Kitamura M (2009) Acquisition of anergy to proinflammatory cytokines in non-immune cells through endoplasmic reticulum stress response: a mechanism for subsidence of inflammation. J Immunol 182:1182–1191

Hayakawa K, Nakajima S, Hiramatsu N, Okamura M, Huang T, Saito Y, Tagawa Y, Tamai M, Takahashi S, Yao J, Kitamura M (2010) ER stress depresses NF-κB activation in mesangial cells through preferential induction of C/EBPβ. J Am Soc Nephrol 21:73–81

Haynes CM, Titus EA, Cooper AA (2004) Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. Mol Cell 15:767–776

Hiramatsu N, Kasai A, Hayakawa K, Yao J, Kitamura M (2006) Real-time detection and continuous monitoring of ER stress in vitro and in vivo by ES-TRAP: evidence for systemic, transient ER stress during endotoxemia. Nucleic Acids Res 34:e93

Hiramatsu N, Kasai A, Du S, Takeda M, Hayakawa K, Okamura M, Yao J, Kitamura M (2007) Rapid, transient induction of ER stress in the liver and kidney after acute exposure to heavy metal: evidence from transgenic sensor mice. FEBS Lett 581:2055–2059

Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH (2006) Autocrine tumor necrosis factor-α links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1α-mediated NF-κB activation and down-regulation of TRAF2 expression. Mol Cell Biol 26:3071–3084

Ishido M, Homma-Takeda S, Tohyama C, Suzuki T (1998) Apoptosis in rat renal proximal tubular cells induced by cadmium. J Toxicol Environ Health A 55:1–12

Jacobson MD (1996) Reactive oxygen species and programmed cell death. Trends Biochem Sci 21:83–86

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

Kim R, Emi M, Tanabe K, Murakami S (2006) Role of the unfolded protein response in cell death. Apoptosis 11:5–13

Kitamura M (2008) Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: janus faces. Am J Physiol Renal 295:F323–F342

Kitamura M (2009) Biphasic, bidirectional regulation of NF-κB by endoplasmic reticulum stress. Antioxid Redox Signal 11:2353–2364

Kwon OY, Kim YJ, Choi Y, Kim H, Song C, Shong M (1999) The endoplasmic reticulum chaperone GRP94 is induced in the thyrocytes by cadmium. Z Naturforsch C 54:573–577

Lee AS (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 26:504–510

Lind Y, Engman J, Jorhem L, Glynn AW (1997) Cadmium accumulation in liver and kidney of mice exposed to the same weekly cadmium dose continuously or once a week. Food Chem Toxicol 35:891–895

Liu F, Inageda K, Nishitai G, Matsuoka M (2006) Cadmium induces the expression of Grp78, an endoplasmic reticulum molecular chaperone, in LLC-PK1 renal epithelial cells. Environ Health Perspect 114:859–864

Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, Kadiiska MB (2008) Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies. Free Radic Biol Med 45:475–481

Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238:209–214

Malhotra JD, Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Signal 9:2277–22793

McKnight SL (2001) McBindall: a better name for CCAAT/enhancer binding proteins? Cell 107:259–261

Nogawa K (1981) Itai–itai disease and follow-up studies. In: Nriagu JO (ed) Cadmium in the environment. Wiley, New York, pp 1–37

Okamura M, Takano Y, Hiramatsu N, Hayakawa K, Yao J, Paton AW, Paton JC, Kitamura M (2008) Suppression of cytokine responses by indomethacin in podocytes: a mechanism through induction of unfolded protein response. Am J Physiol Renal Physiol 295:F1495–F1503

Organization World Health (1992) Cadmium. Environmental health criteria. World Health Organization, Geneva

Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ, Hotamisligil GS (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313:1137–1140

Pahl HL, Baeuerle PA (1995) A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-κB. EMBO J 14:2580–2588

Pourova J, Kottova M, Voprsalova M, Pour M (2010) Reactive oxygen and nitrogen species in normal physiological processes. Acta Physiol (Oxf) 198:15–35

Prozialeck WC, Lamar PC (1995) Effects of glutathione depletion on the cytotoxic actions of cadmium in LLC-PK1 cells. Toxicol Appl Pharmacol 134:285–295

Qian Y, Falahatpisheh MH, Zheng Y, Ramos KS, Tiffany-Castiglioni E (2001) Induction of 78 kD glucose-regulated protein (GRP78) expression and redox-regulated transcription factor activity by lead and mercury in C6 rat glioma cells. Neurotox Res 3:581–589

Rodrigues CM, Sola S, Nan Z, Castro RE, Ribeiro PS, Low WC, Steer CJ (2003) Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. Proc Natl Acad Sci USA 100:6087–6092

Sekine Y, Takeda K, Ichijo H (2006) The ASK1-MAP kinase signaling in ER stress and neurodegenerative diseases. Curr Mol Med 6:87–97

Stein B, Cogswell PC, Baldwin AS Jr (1993) Functional and physical associations between NF-κB and C/EBP family members: a Rel domain—bZIP interaction. Mol Cell Biol 13:3964–3974

Szabo C, Ohshima H (1997) DNA damage induced by peroxynitrite: subsequent biological effects. Nitric Oxide 1:373–385

Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J, Kitamura M (2008) Induction of apoptosis by cigarette smoke via ROS-dependent ER stress and CHOP. Free Radic Biol Med 45:50–59

Takano Y, Hiramatsu N, Okamura M, Hayakawa K, Shimada T, Kasai A, Yokouchi M, Shitamura A, Yao J, Paton AW, Paton JC, Kitamura M (2007) Suppression of cytokine response by GATA inhibitor K-7174 via unfolded protein response. Biochem Biophys Res Commun 360:470–475

Tchounwou PB, Ishaque AB, Schneider J (2001) Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Mol Cell Biochem 222:21–28

Thevenod F (2003) Nephrotoxicity and the proximal tubule. Insights from cadmium. Nephron Physiol 93:87–93

Thevenod F, Friedmann JM, Katsen AD, Hauser IA (2000) Up-regulation of multidrug resistance P-glycoprotein via NF-κB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. J Biol Chem 275:1887–1896

Timblin CR, Janssen YM, Goldberg JL, Mossman BT (1998) GRP78, HSP72/73, and cJun stress protein levels in lung epithelial cells exposed to asbestos, cadmium, or H_2O_2. Free Radic Biol Med 24:632–642

Wang SH, Shih YL, Lee CC, Chen WL, Lin CJ, Lin YS, Wu KH, Shih CM (2009) The role of endoplasmic reticulum in cadmium-induced mesangial cell apoptosis. Chem Biol Interact 181:45–51

Weber M, Sydlik C, Quirling M, Nothdurfter C, Zwergal A, Heiss P, Bell S, Neumeier D, Ziegler-Heitbrock HWL, Brand K (2003) Transcriptional inhibition of interleukin-8 expression in tumor necrosis factor-tolerant cells: evidence for involvement of C/EBP. J Biol Chem 278:23586–23593

Wu J, Kaufman RJ (2006) From acute ER stress to physiological roles of the unfolded protein response. Cell Death Differ 13:374–384

Xie J, Shaikh ZA (2006) Cadmium-induced apoptosis in rat kidney epithelial cells involves modulation of NF-κB activity. Toxicol Sci 224:56–65

Xie Z, Zhang Y, Li A, Li P, Ji W, Huang D (2010) Cd-induced apoptosis was mediated by the release of Ca^{2+} from intracellular Ca storage.Toxicol Lett 192:115–118

Xue X, Piao JH, Nakajima A, Sakon-Komazawa S, Kojima Y, Mori K, Yagita H, Okumura K, Harding H, Nakano H (2005) Tumor necrosis factor-α (TNF-α) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNF-α. J Biol Chem 280:33917–33925

Yeh JH, Huang CC, Yeh MY, Wang JS, Lee JK, Jan CR (2009) Cadmium-induced cytosolic Ca^{2+} elevation and subsequent apoptosis in renal tubular cells. Basic Clin Pharmacol Toxicol 104:345–351

Yokouchi M, Hiramatsu N, Hayakawa K, Kasai A, Takano Y, Yao J, Kitamura M (2007) Atypical, bidirectional regulation of cadmium-induced apoptosis via distinct signaling of unfolded protein response. Cell Death Differ 14:1467–1474

Yokouchi M, Hiramatsu N, Hayakawa K, Okamura M, Du S, Kasai A, Takano Y, Shitamura A, Shimada T, Yao J, Kitamura M (2008) Involvement of selective reactive oxygen species upstream of proapoptotic branches of unfolded protein response. J Biol Chem 283:4252–4260