Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces

Masanori Kitamura
Department of Molecular Signaling, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

Kitamura M. Endoplasmic reticulum stress and unfolded protein response in renal pathophysiology: Janus faces. Am J Physiol Renal Physiol 295: F323–F334, 2008. First published March 26, 2008; doi:10.1152/ajprenal.00050.2008.—A number of pathophysiological insults lead to accumulation of unfolded proteins in the endoplasmic reticulum (ER) and cause ER stress. In response to accumulation of unfolded/misfolded proteins, cells adapt themselves to the stress condition via the unfolded protein response (UPR). For the cells, UPR is a double-edged sword. It triggers both prosurvival and proapoptotic signals. ER stress and UPR may, therefore, be involved in a diverse range of pathological situations. However, currently, information is limited regarding roles of ER stress and UPR in the renal pathophysiology. This review describes current knowledge on the relationship between ER stress and diseases and summarizes evidence for the link between ER stress/UPR and renal diseases.

UPR and Cell Fate

ER stress is defined as accumulation of unfolded or misfolded proteins in the ER, which induces a coordinated adaptive program, 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 (S1P) and site-2 protease (S2P), yielding an active transcription factor, 50-kDa ATF6 (p50ATF6). Similarly, activated IRE1 catalyzes removal of a small intron from the mRNA of the gene encoding 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 an ER chaperone 78-kDa glucose-regulated protein (GRP78; also called Bip) and ERAD factors involved in degradation of unfolded proteins. These pathways are generally regarded as prosurvival UPR (120) (Fig. 1).

During the UPR, however, death signals, as well as survival signals, may also be transduced (50). For example, activation of the PERK-eIF2α pathway causes general inhibition of translation, whereas selectively induces a transcription factor ATF4. Consequently, ATF4 induces expression of proapoptotic CCAAT/enhancer-binding protein-homologous protein (CHOP) [also called growth arrest and DNA damage-inducible protein 153 (GADD153)] through activation of the amino acid response element (AARE). The ATF6 pathway and the IRE1 pathway may also induce expression of CHOP (64). ER stress...
activates caspase-12 (or caspase-4 in humans) localized at the ER membrane through an interaction with IRE1 and tumor necrosis factor receptor-associated factor 2 (TRAF2), leading cells to undergo apoptosis. The IRE1-TRAF2 interaction also allows for recruitment and activation of apoptosis signal-regulating kinase 1 (ASK1) and downstream c-Jun N-terminal kinase (JNK), which is involved in a variety of proapoptotic signaling (50). These pathways have been considered as proapoptotic UPR (Fig. 2).

The three UPR branches initiated by PERK, ATF6, and IRE1 can be involved in prosurvival and proapoptotic signaling. How the UPR integrates its cytoprotective and proapoptotic outputs to select cell fates is unknown. Recently, however, Lin et al. (57) suggested a model by which distinct combinations of individual UPR determine cell fates during ER stress. They showed that activity of IRE1 and ATF6 is attenuated by persistent ER stress. In contrast, PERK signaling, including translational inhibition and induction of CHOP, is maintained even in the chronic phase. When IRE1 activity is sustained artificially, cell survival is enhanced, suggesting a causal link between cell fates and duration of UPR (57). In this model, the initial combined activation of PERK, ATF6, and IRE1 produces cytoprotective outputs such as reduced translation, enhanced protein folding capacity in the ER, and clearance of unfolded proteins, along with proapoptotic outputs such as induction of CHOP. Cytoprotective outputs outweigh proapoptotic outputs in this early phase. This phase provides a “window of opportunity” for cells to readjust their ER to cope with stress. If these steps fail to reestablish homeostasis, IRE1 signaling and ATF6 signaling are attenuated, resulting in an imbalance in which proapoptotic outputs outweigh prosurvival outputs (57).

**Evaluation of ER Stress**

Evaluation and monitoring of ER stress are required for investigation of molecular events involved in ER stress-related pathophysiologies. Based on the above-mentioned knowledge on UPR, several methods have been used for assessment of ER stress. Expression of endogenous biomarkers, e.g., GRP78 and CHOP, is most commonly used for this purpose (52, 64). Phosphorylation of PERK and eIF2α, cleavage of ATF6 and
procaspase-12, and splicing of XBP-1 mRNA have also been used as endogenous indicators for ER stress (72, 104). Alternatively, reporter assays using the ERSE combined with lacZ or luciferase have been used for monitoring ER stress (27, 66). However, these systems require extraction of RNA or protein and do not allow for continuous or successive monitoring of ER stress in living cells and animals. Use of green fluorescence proteins may be useful for this purpose (38), but it is still not competent for quantitative, successive assessment of ER stress in internal organs of living animals (133).

Another means to evaluate ER stress is through the use of a secreted reporter protein. Secretory proteins enter the subcellular pathway through the ER. In the ER, the proteins are folded into native conformation and undergo a multitude of posttranslational modifications. Only correctly folded proteins may be useful for this purpose (38), but it is still not competent for quantitative, successive assessment of ER stress in internal organs of living animals (133).

Another means to evaluate ER stress is through the use of a secreted reporter protein. Secretory proteins enter the subcellular pathway through the ER. In the ER, the proteins are folded into native conformation and undergo a multitude of posttranslational modifications. Only correctly folded proteins are exported to the Golgi apparatus. Based on this current knowledge, perturbation of ER function (i.e., ER stress) can be monitored using certain secreted reporter proteins. We recently reported that ER stress-responsive secreted alkaline phosphatase (ES-TRAP) serves as a sensitive, quantitative biomarker for ER stress (30). In vitro, activity of ES-TRAP secreted by transfected cells is attenuated in response to ER stress independent of its transcriptional regulation. This phenomenon is observed in a wide range of cell types triggered by various ER stress inducers with high sensitivity and selectivity (30). We also generated transgenic mice systemically expressing ES-TRAP and provided evidence for its usefulness for real-time monitoring of ER stress in animals (29, 30). Using these in vitro and in vivo systems, it is feasible to monitor ER stress continuously in living cells and animals by simple sampling of culture medium or small amounts of serum (~5 μl). Recently, similar systems have been developed for continuous monitoring of ER stress in culture cells using secreted luciferases as indicators (5, 28). It is based on the fact that, like ES-TRAP, extracellular activity of secreted luciferase is markedly suppressed under ER stress conditions. However, in contrast to ES-TRAP, activity of secreted luciferase is significantly interfered in the presence of serum, especially serum albumin, as we previously reported (31). The secreted luciferase-based system may not be applicable for in vivo evaluation of ER stress.

Dark Side of UPR

**UPR and apoptosis.** UPR is known to trigger several pathways to apoptosis. The first is the Ca^{2+}-mediated signaling pathway. ER stress causes conformational changes and/or oligomerization of proapoptotic Bak and Bax at the ER mem-
brane (137), leading to release of Ca\(^{2+}\) from the ER. It activates calpain in the cytosol, which cleaves procaspase-12 to mature caspase-12 in the ER (85) (Fig. 2). Activated caspase-12 then initiates a caspase cascade through cleavage of procaspase-9 and procaspase-3 and causes consequent apoptosis (70). Downregulation of Bcl-2 transcription by ER stress-induced CHOP may also be involved in the induction of apoptosis (67). Of note, CHOP-deficient cells are resistant to ER stress-induced apoptosis (136). Another important pathway involved in the ER stress-initiated apoptosis is the IRE1-TRAF2-ASK1-JNK pathway. Indeed, IRE1α-deficient fibroblasts are impaired in JNK activation by ER stress, and dominant-negative TRAF2 inhibits activation of JNK by IRE1 (115). In this pathway, formation of an IRE1-TRAF2-ASK1 complex is essential for activation of JNK by ER stress (76).

**UPR and activation of mitogen-activated protein kinases and NF-κB.** In general, ER stress is known to activate mitogen-activated protein (MAP) kinases and NF-κB and thereby to induce cellular activation. UPR has the ability to activate stress kinases including JNK and p38 MAP kinase via the IRE1-ASK1 pathway (105). Similarly, UPR activates NF-κB through multiple mechanisms, possibly, via the IRE1 pathway (34, 46) and/or the PERK-eIF2α pathway (13, 40). For example, in response to ER stress, IkB kinase (IKK) forms a complex with IRE1α through the adapter protein TRAF2, and ER stress-induced activation of NF-κB is impaired by knockdown or knockout of either IRE1α or TRAF2 (34, 46). Other reports also showed that phosphorylation of eIF2α is necessary and sufficient to activate NF-κB (13, 40), but the molecular mechanisms involved have not been fully elucidated.

**Light Side of UPR**

**UPR and cell survival.** As described, in response to ER stress, cells adapt themselves to the stress condition via attenuation of general translation, induction of ER chaperones and foldases, and activation of ERAD to eliminate immature proteins. ER stress causes induction of the PERK-eIF2α pathway, resulting in general inhibition of translation. In response to ER stress, activated ATF6 and XBP1 bind to ERSE and/or UPRE, leading to expression of ER chaperones including GRP78 and induction of ERAD factors. These pathways contribute to survival of cells under ER stress conditions (50).

**UPR and cell differentiation.** UPR provides not only pro-survival signals but also a wide spectrum of physiological signals. Individual branches of UPR have specialized roles in developmental and metabolic processes. For example, UPR is required during differentiation of some professional cells including plasma cells, pancreatic β cells, hepatocytes, osteoblasts, and myocytes (74, 120).

**PLASMA CELL.** During differentiation of B lymphocytes into plasma cells, UPR drives biogenesis of the ER and takes an essential part in the differentiation of B cells (100). In particular, the IRE1-XBP1 branch of UPR plays a crucial role. It is based on the facts that 1) IRE1-deficient B cells do not differentiate into plasma cells in vitro (134), 2) XBP1-deficient B cells fail to differentiate into plasma cells in vivo (98), and 3) ectopic expression of the spliced form of XBP1 restores immunoglobulin production in XBP1-deficient B cells in vitro (37).

**PANCREATIC β CELL.** PERK is an eIF2 protein kinase highly expressed in the pancreas. Deletion of PERK results in progressive loss of pancreatic β cells in both mice and humans (12, 23). PERK-deficient mice show defect in β cells and develop diabetes (23). In humans, mutations in the PERK gene cause Wolcott-Rallison syndrome which manifests as an infantile-onset, insulin-requiring diabetes (12). Furthermore, eIF2α knock-in mice in which all eIF2 kinases are dysregulated develop severe β cell dysfunction before birth (103).

**HEPATOCYTE.** Both IRE1- and XBP1-deficient mice exhibit hypoplastic fetal livers. In XBP1-null hepatocytes, cell growth is severely affected, and apoptosis is accelerated (97). PERK-deficient mice also show defect in hepatocytes (23), indicating that multiple branches of UPR are required for normal development of hepatocytes.

**OSTEOBLAST.** ATF4 induced by the PERK-eIF2α pathway regulates the onset of osteoblast differentiation, type I collagen synthesis, osteoblast-specific gene expression, and terminal differentiation of osteoblasts (125). Mice and humans deficient in the PERK gene have same abnormality of the bone trabeculae as that in ATF4-deficient mice (12, 135).

**MYOCYTE.** A previous report demonstrated activation of caspase-12 and induction of GRP78 and CHOP during differentiation of C2C12 myoblasts into myocytes (74). In this process, ATF6, but not other ER stress transducers, is activated and contributes to myocyte differentiation. Furthermore, induction of ER stress by tunicamycin or thapsigargin enhances myofiber formation (73), confirming the role of UPR in the differentiation of myoblasts.

**ER Stress and Pathologies**

**Neurodegenerative disorder.** Neurons are sensitive to protein aggregates, and a number of previous reports suggested that ER stress is involved in neurodegenerative disorders (18). For example, ER stress is caused in the brain of patients with Alzheimer’s disease, and PERK and caspase-4 are activated in the onset of this disease (33, 84). Parkinson’s disease is another extensively studied neurodegenerative disorder that is characterized by a loss of dopaminergic neurons. Several previous reports suggested the link between ER stress and Parkinson’s disease. For example, expression of ER chaperones is upregulated in the brain of patients with this disease (9), and Parkinsonism-inducing neurotoxins 6-hydroxydopamine and 1-methyl-4-phenylpyridinium trigger UPR and consequent death of dopaminergic neurons (32). Involvement of ER stress in the pathogenesis of other neurodegenerative disorders has also been proposed by some investigators. Those include polyglutamine diseases, prion diseases, amyotrophic lateral sclerosis, and GM1 gangliosidosis (132).

**Diabetes mellitus.** Type 1 diabetes is the most common autoimmune disease affecting almost 20 million people worldwide. This disease is thought to be caused by destruction of pancreatic β cells by autoactive T cells. The link between ER stress and type 1 diabetes mellitus has been suggested by several investigators. For example, in Akita diabetic mice, ATF6 and XBP1 pathways are constitutively active in pancreatic β cells (78). PERK-deficient mice show symptoms characteristic of type 1 diabetes mellitus (23), and mice with a homozygous mutation at the eIF2α phosphorylation site show...
the similar pathological feature (102, 135). Furthermore, the onset of diabetes is delayed in CHOP-deficient mice (87).

ER stress may also be involved in type 2 diabetes mellitus. The insulin resistance and hyperglycemia in type 2 diabetes are accommodated by an increase in proinsulin translation. Under this condition, UPR is activated, and prolonged activation of UPR may cause β cell death. A previous report showed that overexpression of 150-kDa oxygen-regulated protein (ORP150), an ER chaperone, in the liver of obese diabetic mice significantly improves insulin resistance and ameliorates glucose tolerance. Conversely, expression of antisense ORP150 in the liver of normal mice decreases insulin sensitivity, suggesting that ER stress plays a crucial role in the insulin resistance in type 2 diabetes (75). Consistent with this result, a recent report suggested usefulness of chemical chaperones for the treatment of type 2 diabetes (91). In this report, the authors showed that treatment of obese and diabetic mice with 4-phenylbutyrate (4-PBA), a chemical chaperone that stabilizes protein conformation and improves folding capacity of the ER, resulted in normalization of hyperglycemia, restoration of systemic insulin sensitivity, resolution of fatty liver disease, and enhancement of insulin action in liver, muscle, and adipose tissues (91).

Ischemic injury. Hypoxia and nutritional deprivation cause ER stress. Because of this reason, ER stress is an important pathogenic factor that triggers ischemic injury. For example, cerebral ischemia induces ER stress in neurons and activates UPR including the ATF6, IRE1, and PERK pathways (99), leading to CHOP-mediated apoptosis of neurons (108). Ischemia also induces ER stress in the heart, leading to degeneration of cardiomyocytes (4). Previous reports showed that overexpression of ORP150 ameliorates ischemic injury of several organs including the brain, kidney, and heart (2, 6, 110).

Cancer. Hypoxia is a common feature of malignant tumors with resistance to therapy and poor prognosis (17). Hypoxia induces ER chaperones including GRP78 and GRP94 (56). Upregulation of ER chaperones is observed in a variety of tumors, and expression of ER chaperones increases resistance of tumor cells to chemotherapeutic agents. Conversely, antisense-mediated inhibition of GRP78 sensitizes tumors to hypoxia, and in vivo inhibition of GRP78 also attenuates growth of tumors (17). In addition, UPR also induces the multidrug resistance gene MDR and thereby mediates drug resistance of tumor cells (55).

Autophagy is a lysosomal catabolic process involved in recycling of cellular components and maintenance of cellular homeostasis. Induction of autophagy may be linked to tumor survival by supporting its adaptation to hypoxic and malnutritional microenvironments (62). Recent reports elucidated that ER stress can induce autophagy (79, 131). This process may be another possible mechanism by which tumor cells acquire resistance to apoptosis through UPR.

UPR may also contribute to tumor growth in other ways, e.g., through induction of angiogenesis. Some previous reports showed that ER stress triggers expression of vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis under hypoxia (1, 90). ER stress-inducible ORP150 facilitates correct processing and secretion of VEGF (89, 90). UPR thus contributes to tumor growth not only via protection of tumor cells from apoptosis but also via VEGF-mediated promotion of angiogenesis.

Atherosclerosis. One of the risk factors for atherosclerosis is accumulation of homocysteine, an intermediate product during the metabolism of sulfur amino acids. Previous reports showed that homocysteine induces ER stress that activates sterol regulatory element-binding proteins (SREBP1s) in vascular endothelial cells and smooth muscle cells (118). Activation of SREBP1s causes increased expression of genes responsible for biosynthesis and intracellular accumulation of cholesterol/triglyceride. The homocysteine-triggered gene expression is inhibited by overexpression of ER chaperone GRP78 (118), indicating a direct role of ER stress in the activation of the cholesterol/triglyceride biosynthesis and acceleration of atherosclerosis.

Inflammation. ER stress has the potential to activate NF-κB in the acute phase (92). As described, UPR triggers activation of NF-κB through the IRE1-TRAF2 pathway (34, 46) and/or the PERK-eIF2α pathway (13, 40). It is therefore not surprising that ER stress causes some types of inflammation. A recent report showed that intestinal epithelial cells from mice and humans with inflammatory bowel diseases exhibit activation of UPR evidenced by upregulation of GRP78. The authors reported that increased GRP78 plays a crucial role in the activation of NF-κB via binding to IKK (107). ER stress may also be involved in autoimmune inflammation including rheumatoid arthritis and autoimmune myositis (71, 124). Recently, we reported that systemic inflammatory responses caused by lipopolysaccharide are associated with upregulation of GRP78 in various organs including the lung, liver, kidney, spleen, and heart (30).

Viral infection. Infection by a wide range of viruses including hepatitis B virus, hepatitis C virus, hepatitis D virus, flavivirus, Borna disease virus, and Moloney murine leukemia virus induces ER stress, possibly because of synthesis of various viral proteins (132). UPR triggered by viral infection may contribute to local inflammation and facilitate the cells to undergo apoptosis, leading to hepatic injury, carcinogenesis, and neurodegeneration (132).

Mechanical stress-induced injury. It has been reported that ER stress may be induced in mechanical stress-related pathologies. For example, pressure overload by aortic constriction induces expression of ER chaperones and ER stress-induced apoptosis of cardiomyocytes in vivo (82). The similar pathological mechanism may also be involved in the injury of the urinary bladder suffered from outlet obstruction. We recently reported that outlet obstruction induces expression of ER stress markers, GRP78 and CHOP, in the bladder. It was associated with induction of markers for mechanical stress and hypoxia. In the bladder subjected to outlet obstruction, apoptosis was induced in the epithelial cells and smooth muscle cells, and in vivo administration with a chemical chaperone 4-PBA significantly attenuated apoptosis in the bladder (101).

Bipolar disorder. Bipolar disorder is a common mood disease in which patients have recurrent episodes of mania and depression. Recent investigations suggested possible involvement of UPR in this disease. Genetic linkage studies and microarray analysis indicated that aberrant expression and/or function of XBP1 and GRP78 may be a risk factor for bipolar disorder (43, 44). Interestingly, mood-stabilizing drugs popularly used for the treatment of bipolar disorder can correct impaired function of XBP1 and increase expression of ER chaperones including GRP78 and GRP94 (44, 106).
**ER Stress and Kidney Disease**

**Glomerular disease.** Glomerular diseases are developed by various causes, but little is known about involvement of ER stress in glomerular injury. However, recent investigations suggest roles of ER stress in some types of glomerular disorders, especially proteinuric diseases caused by injury of podocytes.

**CONGENITAL NEPHROTIC SYNDROME.** Congenital nephrotic syndrome of the Finnish type (CNF) is an autosomal recessive disorder characterized by massive proteinuria. The gene responsible for this disease encodes a podocyte-specific membrane protein, nephrin, the principal component of the slit diaphragm (49). More than 60 different mutations have been identified in patients with CNF, and the most common mutations are missense mutations resulting in single amino acid substitutions. A previous study showed that the majority of the missense mutations lead to protein misfolding and consequent retention of the mutants in the ER (60). Liu et al. (61) reported that a chemical chaperone 4-PBA rescues the mutants of nephrin to be transported to the plasma membrane to function similarly to the wild-type nephrin.

Mutation in podocin, a prohibit homology domain protein that is also localized at the slit diaphragm, is another common cause of hereditary nephrotic syndrome in humans. The NPHS2 gene encoding podocin is linked to the autosomal recessive type of steroid-resistant nephrotic syndrome. Ohashi et al. (80) reported that the R138Q mutant of podocin, one of the most common missense mutations in the NPHS2 gene, is retained in the ER, suggesting that trafficking of the mutant podocin is disturbed. Treatment of the cells with chemical chaperones including glycerol, trimethylamine-N-oxide, and dimethylsulfoxide elicits cellular redistribution of R138Q podocin to the plasma membrane (80).

These results suggest a role of ER stress in some congenital nephrotic syndrome. Interestingly, Fujii et al. (19) reported that glucocorticoid, the most popular therapeutic agent for nephrosis, may exert an anti-proteinuric effect via facilitation of intracellular trafficking of nephrin under an ER stress condition. They found that glucose starvation evokes ER stress and formation of hypoglycosylated nephrin that is retained in the ER. Dexamethasone rescues the impaired trafficking and promotes synthesis of fully glycosylated nephrin.

**MEMBRANOUS NEPHROPATHY.** Passive Heymann nephritis in rodents is a model of membranous nephropathy in humans. In this model, complement C5b-9 induces injury of glomerular podocytes, resulting in proteinuria. Cybulsky et al. (11) reported that exposure of cultured podocytes to C5b-9 increases GRP78 and GRP94 mRNAs and proteins. Knockdown of GRP78 via antisense GRP78 enhances complement-dependent injury of cultured podocytes. In vivo, glomerular GRP78 and GRP94 proteins are upregulated in proteinuric rats with passive Heymann nephritis, and pretreatment of the rats with tunica-mycin to induce GRP78 and GRP94 reduces proteinuria (11). The same group also showed that complements induce phosphorylation of PERK and eIF2α and that PERK and eIF2α phosphorylation is enhanced in glomeruli of rats with Heymann nephritis. Fibroblasts from PERK-deficient mice are more susceptible to complement-mediated cytotoxicity, suggesting a prosurvival role of the PERK-eIF2α branch of UPR (10). These results suggested that complement-induced podocyte injury in vitro and in vivo is associated, possibly mediated by ER stress, and that ER stress may be involved in the pathogenesis of membranous nephropathy. Using immunohistochemical staining, Bek et al. (7) recently reported upregulation of CHOP in podocytes of proteinuric human kidneys (membranous nephropathy, focal segmental glomerulosclerosis, and minimal change nephropathy) as well as kidneys of rats with puromycin nephrosis, a model of minimal change nephropathy.

**ISCHEMIC INJURY.** Using an in vitro model of ischemia-reperfusion (2-deoxyglucose plus antimycin A followed by glucose re-exposure), Cybulsky et al. (10) reported that podocytes subjected to ischemia-reperfusion exhibit phosphorylation of PERK and eIF2α. They also found that PERK-deficient fibroblasts are more susceptible to ischemia-reperfusion-triggered cellular death, indicating an anti-apoptotic role of the PERK-eIF2α branch of UPR in ischemic injury of podocytes.

**HOMOCYSTEINE-INDUCED INJURY.** Hyperhomocysteinemia is regarded as a critical risk factor in the progression to end-stage renal diseases. Recent reports suggested a possibility that hyperhomocysteinemia may directly act on glomerular cells to induce glomerular dysfunction and consequent glomerulosclerosis (128). For instance, Yi et al. (127) reported progression of podocyte injury and glomerulosclerosis in hyperhomocysteinemic rats. It is known that ER stress is considered as an important mechanism that causes several pathologies, especially vascular injury, in hyperhomocysteinemia (86, 118). ER stress caused by hyperhomocysteinemia is possibly involved in glomerular injury and glomerulosclerosis.

**Tubular disease.** Acute renal failure is caused by a variety of triggers including hypoxia/ischemia, heavy metal intoxication, sepsis, and nephrotoxic agents such as antibiotics, antitumor agents, immunosuppressants, and nonsteroidal anti-inflammatory drugs (NSAIDs). In general, acute renal failure is characterized by tubular cell damage, apoptosis, and/or necrosis. In particular, apoptosis is thought to play a prominent role in the development of tubular injury during acute renal failure (114).

**ISCHEMIC INJURY.** Montie et al. (68) reported that cardiac arrest-induced ischemia and subsequent reperfusion cause phosphorylation of PERK and eIF2α in the rat kidney, especially in tubular epithelial cells. This observation indicates that renal ischemia-reperfusion induces ER stress, activates UPR, and causes cellular damage in the renal tubules. Bando et al. (6) provided another evidence for the crucial role of ER stress in renal ischemic injury. They found that renal tissues from patients with acute renal failure display strong induction of the ER chaperone ORP150. In a rodent model of renal ischemia-reperfusion injury, ORP150 is induced in the kidney, principally in the renal tubules. Cultured tubular cells exposed to hypoxia display induction of ORP150. Tubular cells transfected with ORP150 exhibit resistance to hypoxic stress, whereas knockdown of ORP150 by antisense ORP150 makes the cells susceptible to hypoxic cell death (6). Furthermore, transgenic mice overexpressing ORP150 are resistant to renal ischemia-reperfusion injury. In contrast, mice with lower levels of ORP150 show enhanced ischemic injury (6). These data suggest a key role for ER stress in the ischemic renal tubular injury.

**HEAVY METAL INTOXICATION.** Several heavy metals induce renal tubular injury. One of the most famous examples is the nephrotoxicity of cadmium. Environmental exposures to cad-
mum via drinking water, foods, and cigarette smoke cause accumulation of this metal in a variety of organs, especially in the kidney (36). A typical example is Itai-itai disease in Japan, in which cadmium poisoning was caused via prolonged ingestion of industrially polluted water and rice. A characteristic clinical feature of Itai-itai disease is renal insufficiency manifested by tubular injury and dysfunction (77). Previous investigations suggested that toxic effects of cadmium on the tubules are caused through several mechanisms, e.g., structural alterations in junctional complexes (disruption of tight and gap junctions) and cellular death caused by oxidative stress (39, 111). In addition to these mechanisms, we and others (58, 129) recently reported a pathological role of ER stress in cadmium-induced apoptosis of tubular cells. We found that cadmium elicits ER stress in tubular cells in vitro and in vivo and consequently causes apoptosis. Overexpression of ER chaperone GRP78 or ORP150 suppresses cadmium-triggered apoptosis. In response to cadmium, activation of the PERK-eIF2α pathway, the ATF6 pathway, and the IRE1-XBP1 pathway is induced. In LLC-PK1 cells, the ATF6 pathway and the IRE1 pathway were mainly proapoptotic via induction of CHOP, activation of XBP1, and consequent phosphorylation of JNK. In contrast, the PERK-eIF2α pathway was anti-apoptotic and counteracted the effects of proapoptotic UPR (129). Interestingly, ER stress is triggered not only by cadmium but also by several other nephrotoxic metals (129), indicating a possibility that the similar mechanism is generally involved in metal-induced renal injury.

Previous studies indicated involvement of reactive oxygen species (ROS) in cadmium-induced renal tubular injury. For example, exposure of LLC-PK1 cells to cadmium causes generation of ROS, which is associated with a decrease in glutathione levels and consequent cellular death (20, 96). Another report showed that cadmium-triggered apoptosis of tubular cells is inhibited by an antioxidant (112). Recently, we demonstrated that cadmium-induced ER stress is inhibited by antioxidants (130). In contrast, suppression of ER stress does not attenuate cadmium-triggered oxidative stress, suggesting that ER stress is downstream of oxidative stress (130). We also found that O$_2^-$ is selectively involved in cadmium-triggered, ER stress-mediated apoptosis through activation of the ATF6-CHOP and IRE1-XBP1 pathways (130).

ANTICANCER AGENT. Cisplatin is a chemotherapeutic agent used for the treatment of various solid tumors. Despite its therapeutic effectiveness, its nephrotoxic side effects significantly limit the clinical use. Cisplatin has multiple intracellular effects including direct cytotoxicity via generation of ROS, activation of MAP kinases, and induction of inflammation and fibrogenesis via generation of cytokines (126). In particular, ROS have been considered as important mediators for cisplatin-induced tubular injury. Liu and Baliga (59) showed that, in cisplatin-treated tubular cells, cleavage of procaspase-12 precedes that of caspases-3 and -9. They also showed that transfection with anti-caspase-12 antibody significantly attenuates cisplatin-induced apoptosis. Furthermore, ER stress preconditioning to induce ER chaperones is effective for attenuation of cisplatin cytotoxicity in tubular cell lines including LLC-PK1, NRK-52E, and HEK293 cells (93). These results suggest that ER stress and consequent activation of caspase-12 play a pivotal role in cisplatin-induced nephrotoxicity. Indeed, Peyrou et al. (94) recently provided in vivo evidence for the involvement of ER stress in cisplatin-induced renal injury. They showed that, after administration with cisplatin in rats, activation of the XBP1 pathway and cleavage of procaspase-12 are observed in the kidney.

NSAID. A previous report showed that certain NSAIDs cause ER stress in gastric mucosal cells (113). Recently, we also found that indomethacin, but not other NSAIDs tested, induces ER stress in murine podocytes (83). It is known that NSAIDs exert nephrotoxicity (65), and ER stress may underlie the nephrotoxic effects of NSAIDs. Indeed, Lorz et al. (63) reported that paracetamol (also known as acetaminophen) induces apoptosis of tubular epithelial cells, which is correlated with upregulation of CHOP and cleavage of procaspase-12.

IMMUNOSUPPRESSANT. Calcineurin inhibitors, cyclosporine A (CsA) and tacrolimus (FK506), improve allograft survival in organ transplantation. However, chronic allograft dysfunction is the major hindrance to long-term graft survival, and nephrotoxicity of calcineurin inhibitors may be one of the major factors responsible for chronic allograft dysfunction (119). Justo et al. (42) reported that tubular cell apoptosis induced by CsA is associated with induction of CHOP. We found that, in renal tubular cells, CsA and FK506 cause upregulation of endogenous and exogenous indicators for ER stress. The induction of ER stress by these agents is reversible and observed similarly in several other nonimmune cells. Systemic administration with CsA into mice also causes rapid, significant induction of ER stress in the kidney (16). Furthermore, Peyrou and Cribb (93) reported that ER stress preconditioning is effective for decreasing the toxicity of CsA in tubular cell lines including LLC-PK1, NRK-52E, and HEK293 cells. These results suggest a role of ER stress in the nephrotoxicity of calcineurin inhibitors.

ANTIBIOTIC. Aminoglycosides are major nephrotoxic antibiotics that cause tubular injury. The toxicity of aminoglycosides is related to their uptake by proximal tubular cells and disruption of metabolism of anionic phospholipids, especially phosphoinositides (45). Jin et al. (41) reported that gentamicin causes cleavage of m-calpain and procaspase-12 in NRK cells, indicating involvement of ER stress. Peyrou et al. (93) reported that ER stress preconditioning is effective for decreasing the toxicity of gentamicin in LLC-PK1 cells. They also showed that, after administration with gentamicin in rats, activation of the XBP1 pathway and cleavage of procaspase-12 are induced in the kidney (94).

NEPHROTOXIC CYSTEINE CONJUGATE. Nephrototoxic cysteine conjugate (NCC) such as S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) generates reactive acylating species in the proximal tubules and is used to induce injury of renal tubules in vitro and in vivo. Previous reports showed that ER stress preconditioning prevents NCC-induced toxicity in LLC-PK1 cells. For example, Asmellash et al. (3) reported that ER stress preconditioning protects the proximal tubular cells against a subsequent challenge with TFEC. Furthermore, they also showed that attenuation of GRP78 expression by antisense GRP78 abrogates the protective effect, indicating that ER stress is involved in the NCC-induced injury of renal tubular cells.

PROTEINURIA-ASSOCIATED INJURY. Chronic proteinuria is considered as a pathogenic factor in tubulointerstitial damage. Ohs et al. (81) reported that viability of tubular cells decreases after exposure to bovine serum albumin. It is associated with
induction of ER chaperones including GRP78 and ORP150 and activation of caspase-12. Furthermore, they also demonstrated that upregulation of ER stress indicators as well as activation of caspase-12 are observed in proximal tubules of experimental proteinuric rats. These results indicate that renal tubules exposed to high albumin concentrations exhibit ER stress, which may result in tubular injury.

**AGING-RELATED TUBULOINTERSTITIAL DAMAGE.** Tubulointerstitial damage is observed in a variety of chronic kidney diseases. Kimura et al. (51) generated an ER stress-sensitive knock-in mouse that expresses a mutant GRP78 in which the retrieval sequence to the ER is deleted. The heterozygous transgenic mice showed accelerated tubulointerstitial lesions during aging. Furthermore, tubulointerstitial damage caused by chronic protein overload was also accelerated in the transgenic mice.

**ER Stress and Physiological Event: Renal Development and Hormonal Response**

As described, UPR plays an important role in the development of professional cells including plasma cells, pancreatic β cells, hepatocytes, osteoblasts, and myocytes (102). Currently, information is limited regarding whether and how ER stress and UPR are involved in the renal development, but a previous report showed that expression of GRP78 is induced in developing metanephric kidneys (95). Further investigation will be required to clarify developmental significance of this observation.

Vasopressin regulates water and solute transport in the renal collecting duct. Using a proteomic analysis, van Balkom et al. (116) reported that subcutaneous infusion of vasopressin in Brattleboro rats causes induction of GRP78 in the inner medullary collecting ducts, implying a possible involvement of UPR in the physiological action of vasopressin.

**ER Stress and Oxidative Stress**

We recently reported that ROS including O$_2^-$ and ONOO$^-$ cause ER stress in renal proximal tubular cells (130). Consistent with this result, some previous reports also indicated that ER stress may be involved downstream of oxidative stress. For example, in brain tumor cells, geldanamycin (an inhibitor of 90-kDa heat shock protein) causes expression of GRP78 via a ROS-dependent mechanism (54). In vascular endothelial cells, ONOO$^-$ causes modest increases in GRP78 and GRP94 proteins (15). In the ischemic brain, induction of ATF4 and CHOP is attenuated in transgenic mice and rats overexpressing superoxide dismutase (26). Currently, it is unclear how ROS induce ER stress. Previous reports showed that oxidative stress causes inhibition of Ca$^{2+}$-ATPase (47, 69). One possibility is that ROS cause depletion of calcium stores in the ER via inhibition of Ca$^{2+}$-ATPase (117). Another possibility is that ROS may cause ER stress through generation and accumulation of oxidatively modified, abnormal proteins. Unfolded proteins may also be accumulated in the ER through ROS-induced functional perturbation of ER folds and/or chaperones (8). Interestingly, Hung et al. (35) reported that ER stress preconditioning confers resistance to subsequent H$_2$O$_2$-induced injury in renal tubular epithelial cells. Stable transfection of the cells with antisense RNA targeting GRP78 sensitized the cells to the H$_2$O$_2$-induced injury, suggesting that UPR-induced GRP78 is involved in the self-defense of the cells against oxidative stress. They also found that 1) ERK and JNK are transiently phosphorylated in response to H$_2$O$_2$, 2) ER stress-preconditioned cells have more ERK and less JNK phosphorylation, 3) inhibition of JNK or activation of ERK protected cells against toxicity of H$_2$O$_2$, and 4) antisense RNA targeting GRP78 attenuated ERK activation and JNK phosphorylation following exposure to H$_2$O$_2$ (35). Thus, the ER stress response modulates the balance between the ERK and JNK signaling pathways and may be involved in the regulation of MAP kinases by oxidative stress.

Nitric oxide (NO) is another multifunctional molecule involved in a variety of pathophysiologial processes including regulation of blood vessel dilatation, immune responses, and neurotransmission. It is known that ER stress is caused by NO, and consequent induction of CHOP is involved in NO-induced apoptosis (21). For example, induction of UPR including activation of ATF6 and expression of CHOP is observed in cells exposed to NO, and cells from CHOP-deficient mice show resistance to NO-induced apoptosis (22, 87). Although molecular mechanisms involved have not been fully elucidated, disturbance of Ca$^{2+}$ homeostasis may be implicated in NO-induced ER stress (88, 123). It is because NO inhibits sarco/endoplasmic reticulum Ca$^{2+}$-ATPase by tyrosine nitration within the channel-like domain (122).

ROS and NO are involved in a wide range of cellular events in the kidney under various pathophysiologial circumstances.

**Table 1. Involvement of endoplasmic reticulum stress and unfolded protein response in kidney disease**

<table>
<thead>
<tr>
<th>Disease(s) and Trigger</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glomerular disease</strong></td>
<td></td>
</tr>
<tr>
<td>Congenital nephrotic syndrome</td>
<td>60, 61</td>
</tr>
<tr>
<td>Nephrin gene mutation</td>
<td>80</td>
</tr>
<tr>
<td>Podocin gene mutation</td>
<td></td>
</tr>
<tr>
<td>Primary glomerular disease</td>
<td></td>
</tr>
<tr>
<td>Passive Heymann nephritis</td>
<td>10, 11</td>
</tr>
<tr>
<td>Pyruvycin nephrosis</td>
<td>7</td>
</tr>
<tr>
<td>Proteinuric human glomerular diseases</td>
<td></td>
</tr>
<tr>
<td>Membranoproliferative nephropathy</td>
<td>7</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis</td>
<td>7</td>
</tr>
<tr>
<td>Minimal change nephropathy</td>
<td>7</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion injury</td>
<td>10</td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>127</td>
</tr>
<tr>
<td><strong>Tubular disease</strong></td>
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</tr>
<tr>
<td>Ischemia</td>
<td></td>
</tr>
<tr>
<td>Ischemia-reperfusion</td>
<td>6, 68</td>
</tr>
<tr>
<td>Heavy metal</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>58, 129, 130</td>
</tr>
<tr>
<td>Anticancer agent</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>59, 93, 94, 126</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drug</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>(Unpublished data)</td>
</tr>
<tr>
<td>Paracetamol (acetaminophen)</td>
<td>63</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>16, 42, 93</td>
</tr>
<tr>
<td>Tacrolimus (FK506)</td>
<td>16</td>
</tr>
<tr>
<td>Antibiotic</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>41</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>93, 94</td>
</tr>
<tr>
<td>Nephrotoxic cysteine conjugate</td>
<td></td>
</tr>
<tr>
<td>S-$\alpha$(1,1,2,2-tetrafluoroethyl)-L-cysteine</td>
<td>3</td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
</tr>
<tr>
<td>Chronic proteinuria</td>
<td>81</td>
</tr>
<tr>
<td>Aging</td>
<td></td>
</tr>
<tr>
<td>Tubulointerstitial damage associated with aging</td>
<td>51</td>
</tr>
</tbody>
</table>
The fact that ER stress can be elicited by ROS and NO raises a possibility that ER stress and UPR can also be involved in a variety of renal pathophysiologies.

**ER Stress and Inflammatory Response**

Several previous reports showed that ER stress causes activation of inflammation-related transcription factor NF-κB (13, 34, 40, 46). Under ER stress, IRE1α binds to the IKK complex and activates NF-κB by promoting degradation of IkBs. Moreover, IRE1α binds to TRAF2 and ASK1, leading to activation of IKK (121). Because of this reason, to date, the proinflammatory aspect of ER stress has been emphasized. However, we recently reported that preceding ER stress may blunt subsequent activation of NF-κB. We found that, in glomerular podocytes and mesangial cells, expression of monocyte chemoattractant protein 1 and inducible NO synthase in response to TNF-α is abrogated by UPR-inducing agents K-7174 and geranylgeranylacetone (GGA) (25, 109). The suppression of gene expression by these agents was associated with suppression of NF-κB activation. Induction of UPR by other inducers of ER stress also reproduces the suppressive effects of K-7174 and GGA on NF-κB and NF-κB-dependent genes (25, 109). These results indicated a possibility that, although ER stress activates NF-κB in the early phase, consequent UPR suppresses cellular responses to subsequent inflammatory stimuli in the later phase.

Molecular mechanisms involved in the anti-inflammatory potential of UPR are largely unknown. However, we found that A20, one of major negative regulators for NF-κB, is induced by ER stress, suggesting a possible involvement of this molecule in the blunted responses to inflammatory stimuli under ER stress conditions (24). Currently, it is unclear how A20 is induced by ER stress, but previous reports showed that expression of A20 is regulated by NF-κB (53). The induction of A20 by ER stress may be mediated by the early, transient activation of NF-κB.

In the TNF-α signaling, TNFR receptor 1 (TNFR1), TNFR1-associated death domain (TRADD), TNFR-interacting protein (RIP), and TRAF2 are essential for NF-κB activation. The initial event in TNF-α-induced activation of NF-κB is the ligand-induced formation of a multimeric TNFR1 complex. Formation of this TNF/TNFR1 complex causes its interaction with the adaptor protein TRADD and recruits TRAF2 and RIP into the signaling complex. TRAF2 then recruits the IKK complex, leading to activation of IKK by RIP (14). Recently, Hu et al. (34) reported that, in thapsigargin (inhibitor of sarco/endoplasmic reticulum Ca²⁺-ATPase)- or tunicamycin (inhibitor of protein glycosylation)-treated cells, TNFR1, TRADD, and RIP proteins are maintained at the same levels as those in untreated cells, whereas the level of TRAF2 protein is selectively downregulated. The decrease in TRAF2 protein is not due to transcriptional suppression or increased turnover of mRNA but due to enhanced protein degradation. The blunted responses of NF-κB to TNF-α under ER stress may be caused by downregulation of TRAF2.

Although ER stress may induce proinflammatory molecules via activation NF-κB in the early phase, consequent ER stress response may suppress cellular activation in the later phase. It may play a role in halting progression of acute inflammation and in leading to its subsidence. Currently, molecular mechanisms underlying the “Janus faces” of UPR in the regulation of NF-κB are unclear, but the mechanism for the biphasic regulation of cell fate by UPR, described in the section of UPR and Cell Fate, may also explain the biphasic action of UPR on NF-κB.

**Future Perspective: Unanswered Questions**

Accumulating evidence suggests roles of ER stress and UPR in a wide range of renal pathophysiologies. The current knowledge on the relationship between ER stress and the kidney was summarized in Table 1. However, the majority of previous studies provided only phenomenological evidence, e.g., induction of ER stress markers under particular pathophysiological situations. Extensive investigation will be required to examine how ER stress and UPR contribute to particular renal pathophysiology. ER stress/UPR has Janus faces. It is essential to disclose not only its dark side (pathological significance) but also its light side (developmental and physiological significance) in the kidney. In particular, binary roles of ER stress in inflammatory responses and oxidative stress-induced injury should be clarified in the future. In addition, there are almost no reports regarding roles of ER stress in endothelial function and tubulointerstitial injury in the kidney. Similarly, no information is available regarding kidney-specific ER chaperoning systems. Answering these unanswered questions should be our next targets of investigation.

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