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FORUM REVIEW ARTICLE

Endoplasmic Reticulum Stress and Renal Disease

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Abstract

The accumulation of unfolded proteins in the endoplasmic reticulum (ER), leading to ER stress, is caused by a wide range of physiologic and pathologic conditions. Cells respond to ER stress by activating a series of integrative stress pathways termed the unfolded protein response (UPR). This either may be adaptive and promote cell survival, or if the ER stress is chronic or excessive, may lead to cell death. The role of ER stress in the pathophysiology of both acute and chronic kidney diseases has been gaining increasing interest. This review highlights the current knowledge of ER stress in renal disease, with emphasis on more recent advances. Potential therapeutic options targeting ER stress are discussed. *Antioxid. Redox Signal.* 11, 2341–2352.

Introduction

THE ENDOPLASMIC RETICULUM (ER) is the key intracellular organelle responsible for the synthesis and folding of membrane and secretory proteins. ER stress occurs with an accumulation of unfolded proteins in the ER, which may be due to an unfavorable local environment for protein folding or to mutations in a protein that interferes with its proper folding. ER stress appears to play an important role in a number of renal pathologies, because its induction may lead to cell death and, as such, may be a key regulatory point determining renal cell survival. Apoptosis has been shown to contribute to cell loss in multiple renal compartments, including glomerular podocytes, mesangial cells, and tubular cells (24, 84, 97). In its later stages, chronic renal disease has been considered to be progressive, leading to an inexorable decline in renal function (44). This decline is accompanied by progressive interstitial fibrosis and both glomerular and tubular cell loss (13). Therapeutic interventions that can prevent apoptotic cell death or the conditions leading to it may potentially arrest or slow this decline in renal function.

ER stress and the unfolded protein response

ER stress is a cellular pathology that arises in various conditions or circumstances, or both, that disturb the homeostasis of the ER, preventing it from performing its normal function in the manufacture of secretory and transmembrane proteins (47). Because of the disturbance in ER homeostasis, proteins manufactured in the ER are prevented from attaining their proper tertiary structure and thus are referred to as unfolded proteins. The accumulation of unfolded proteins in the ER under conditions of ER stress results in an evolutionarily conserved cellular response referred to as the unfolded protein response (UPR) (98). Unfolded proteins are prevented from aggregating in the ER and thus impairing ER function by interacting with protein-folding chaperones including glucose-regulated protein 78-KD (GRP78) and glucose-regulated protein 94-KD (GRP94) (98).

GRP78 is currently understood to play a critical role in the regulation of the induction of the UPR, as it is bound to three critical transmembrane proteins found in the ER that constitute the UPR response (Fig. 1). These are activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR (double-stranded RNA-dependent protein kinase)-like ER protein kinase (PERK) (98). The dissociation of GRP78 from these three inducers of the UPR due to its higher affinity for binding with unfolded proteins within the ER lumen leads to inducer activation. In this context, GRP78 has been referred to as a master regulator of the UPR response (57).

ER stress and UPR activation appear to be a general phenomenon occurring in many cell types. However, ER stress may be more physiologically relevant in cell types with a high rate of protein synthesis or the primary function of which is the production of secretory or membrane-resident proteins. For example, insulin-secreting pancreatic β cells require a functional UPR to maintain ER homeostasis and prevent β-cell failure (86). Plasma cells, the primary function of which

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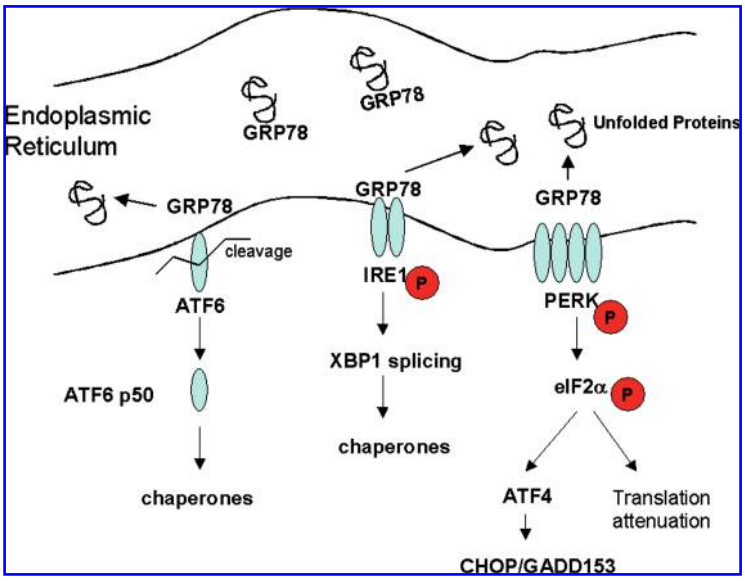

FIG. 1. GRP78 as a master regulator of the unfolded protein response. Endoplasmic reticulum (ER) stress occurs because of a disruption in ER homeostasis, producing an unfavorable environment for the proper folding of membrane or secretory proteins. It may also occur because of mutations in proteins, which prevents their proper folding. The ER chaperone GRP78 is bound to three transmembrane transducers of the unfolded protein response (UPR): activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR-like ER protein kinase (PERK). Dissociation of GRP78 from these transmembrane transducers due to its binding to unfolded proteins within the ER lumen leads to the initiation of the UPR response. This includes (a) phosphorylation of PERK, resulting in eIF2α phosphorylation and attenuation of protein translation; (b) preferential translation of ATF4 and ATF4 induction of CHOP/GADD153, which may lead to apoptosis; (c) phosphorylation of IRE1, resulting in splicing of XBP-1 mRNA and activation of transcription of genes with a UPR response element, including ER chaperones such as GRP78; and (d) translocation and cleavage of ATF6, also resulting in transcription of genes with an ER stress-response element, including ER chaperones, such as GRP78. In this context, GRP78 has been referred to as a master regulator of the UPR because its dissociation from UPR transmembrane transducers initiates the UPR response. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

is the production and secretion of antibodies, require induction of the IRE1 pathway resulting in the splicing of X-box–binding protein 1 (XBP-1) (31, 32). This unique splicing event generates a functional XBP-1 protein that acts as a transcriptional activator to amplify the expression of the ER-resident chaperones GRP78 and GRP94, facilitating differentiation of β cells into plasma cells.

Many ER-resident chaperone proteins, such as GRP78 and GRP94, are constitutively expressed in cells not undergoing ER stress, as they perform necessary functions in protein folding and are not merely stress-response genes. However, under ER stress, the UPR response includes activation of a wide range of cellular pathways that augment cellular survival, including degradation of misfolded proteins, upregulation of the protein expression of constitutively expressed protein-folding chaperones such as GRP78, GRP94, and protein disulfide isomerase (PDI) that increase the protein-folding capacity of the ER, and translation attenuation through eukaryotic translation initiation factor 2 alpha (eIF2α) phosphorylation, which reduces the cellular burden of protein folding. However, prolonged UPR activation has been demonstrated to induce apoptotic cell death, including C/EBP homologous protein/growth arrest and DNA-damage inducible protein 153 (CHOP/GADD153)-mediated β-cell leukemia/lymphoma 2 (Bcl-2) downregulation, T-cell death–associated gene 51 (*TDAG51*) gene induction and caspase-12–induced apoptosis (27, 60, 101). The interaction of these prodeath and prosurvival factors in the cell ultimately determines its fate. Thus, if the protein-folding capacity of the ER can be balanced with the cell requirements *via* adaptation through UPR induction, then the cell can survive and resume its normal function. If, conversely, ER stress cannot be resolved through UPR initiation, prolonged or severe ER stress may result in cellular apoptosis (Fig. 2).

Protein synthesis in the kidney

Protein turnover rates in the kidney are high, and membrane-resident proteins contribute significantly to this synthetic load. Estimated fractional daily protein synthesis rates in the kidney were determined to be ~42% per day in human subjects with leucine and phenylalanine radiotracer infusions. This is in comparison to the splanchnic bed and skeletal muscle, in which rates were ~12% per day and 1.5% per day, respectively (89). In unrelated studies, it was estimated that 30% of renal cortical proteins are turned over each

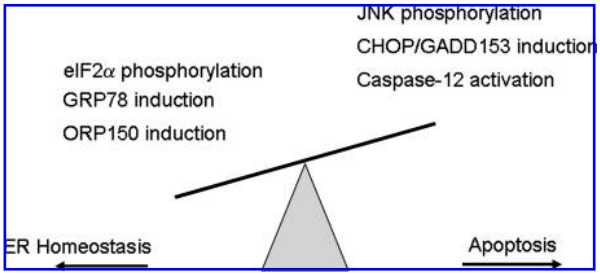

FIG. 2. Elements of the unfolded protein response shown to have prosurvival or proapoptotic effects in kidney disease. The unfolded protein response (UPR) involves effects that may lead to either cell survival or apoptosis, depending on the context. Prosurvival effects include eIF2α phosphorylation, GRP78 induction, and ORP150 induction. Proapoptotic effects of ER stress have been shown to induce or promote renal cell apoptosis and include JNK phosphorylation, CHOP/GADD153 induction, and caspase-12 activation. ER stress–induced apoptosis may play an important role in glomerular and tubular cell loss and progressive decline in renal function associated with chronic kidney disease.

day (19). Glomerular filtration of ~180 L/day of plasma in the normal kidney requires reabsorption of many components of the ultrafiltrate. Membrane-bound transport proteins, channels, and receptors synthesized in the ER facilitate reabsorption of ultrafiltrate components, including glucose, water, amino acids, electrolytes, and other small molecules.

Furthermore, renal protein synthesis has been found to decline with age in the Fischer F344 rat, and this decline is inversely correlated with proteinuria, suggesting that new protein synthesis in the kidney plays a key role in maintaining its function (83). In mice in which GRP78/BiP was mutated to remove its ER retention sequence, although homozygosity was lethal, heterozygotes displayed a progressive decline in renal function with age, characterized by glomerular sclerosis, tubular atrophy and interstitial fibrosis (37, 61).

ER stress in renal pathophysiology

The role of ER stress in the development of renal disease is a relatively recently explored area (38, 39). This review has categorized renal conditions for which the role of ER stress is addressed individually (Table 1), and we highlight renal diseases not yet reviewed by others, including diabetic nephropathy and polycystic kidney disease, as well as consider potential therapeutic options that target ER stress in kidney disease.

Table 1. ER Stress and Kidney Disease

Congenital/hereditary
Congenital nephrotic syndrome
Congenital nephrosis of the Finnish type (CNF) (nephrin mutation, NPHS1)
Autosomal recessive steroid-resistant nephrotic syndrome (Podocin mutation, NPHS2)
Polycystic kidney disease (PCKD)
Proteinuria/albuminuria
Primary glomerular disease
Nonproliferative glomerulonephritis
Minimal-change disease (MCD) (puromycin nephrosis model)
Membranous nephropathy (PHN model)
Focal segmental glomerulosclerosis (FSGS)
Proliferative glomerulonephritis
Membranoproliferative glomerulonephritis (MPGN)
Rapidly progressive/crescentic proliferative glomerulonephritis (RPGN)
Tubular injury
Ischemia/reperfusion
Drugs
Nonsteroidal antiinflammatory drugs (NSAIDs) and prostaglandins
Acetaminophen
Antibiotics (aminoglycosides)
Chemotherapeutics (cisplatin)
Heavy metal–induced injury (cadmium, mercury, lead)
Calcineurin inhibitors (cyclosporin A, tacrolimus)
Kidney transplant
Crystal-induced injury (calcium oxalate)
Other
Diabetic nephropathy
Angiotensin II

Congential/Hereditary

Congenital nephrotic syndrome

The most common causes of congenital nephrotic syndrome are mutations in the genes *NPHS1* or *NPHS2*, encoding the proteins nephrin or podocin, respectively, both of which form an essential component of the glomerular filtration barrier (25). Mutations in nephrin, inherited as an autosomal recessive trait, underlie congenital nephrotic syndrome of the Finnish type (CNF), characterized by severe nephrotic syndrome and kidney failure, usually by age 8 years. Many different mutations in nephrin have been identified, with the majority being missense mutations that result in its retention in the ER, most likely due to protein misfolding (53). Treatment of HEK293 cells overexpressing various mutant nephrin constructs demonstrated that the chemical chaperone sodium 4-phenylbutyrate (4-PBA) was able to rescue the localization defect in several, but not all, of the missense mutants. Relocalization to the plasma membrane was associated with clustering and phosphorylation, suggesting that the rescued mutants may be functional (54).

Autosomal recessive inheritance of podocin mutations leads to steroid-resistant nephrotic syndrome. A study of five common disease-causing missense mutations of podocin identified two that resulted in protein retention in the ER, again suggesting that these mutations interfered with podocin protein folding. Because of the strong association between podocin and nephrin, these mutations also led to abnormal localization of wild-type nephrin in transfected HEK293 cells (68). It was further demonstrated that the chemical chaperones glycerol, DMSO, or trimethylamine-*N*-oxide were able to restore appropriate cellular localization of one of these mutant proteins, although its functionality was not confirmed (70). These studies thus identify a role for protein misfolding and hence mislocalization to the ER in at least some of the disease-causing mutations of congenital nephrotic syndrome. Retention in the ER may, over time, lead to ER stress, although this has not as yet been investigated. More important, however, these findings open an avenue for potential treatment of congenital nephrotic syndrome.

Polycystic kidney disease

Polycystic kidney disease (PKD) is a common autosomal dominant disorder occurring in 1 in 400 to 1 in 1,000 live births in Europe and North America (92). It leads to increasing fluid-filled cyst formation in the tubules over time, with eventual deterioration of renal function. Mutations most commonly occur in either *pkd1* (85–90%) or *pkd2* (10–15%), encoding for polycystins 1 and 2 (PC1 and 2), respectively. Loss-of-function mutations in either gene can lead to the phenotypic expression of PKD. PC1 is an integral membrane glycoprotein that acts as a G protein–coupled receptor, whereas PC2 is a Ca^{2+} channel that, in some reports, was shown to localize to the ER (21, 94). It also was shown to undergo ER-associated degradation (ERAD), a process by which unfolded proteins are delivered into the cytosol for destruction by the 26S ubiquitin-proteasome system (45). PC2 degradation is thus accelerated by ER stress. Furthermore, pathogenic mutations in PC2 have been shown to alter its regulation by the ERAD process (45), and either increased or decreased PC2 expression from the normal state has been associated with disease (91).

Tubular cell proliferation is a fundamental process in the formation of cysts. PC2 was recently shown to downregulate cell proliferation by promoting PERK-mediated eIF2α phosphorylation, which inhibits protein synthesis and cell-cycle progression. Moreover, under conditions of ER stress, knockdown of PC2 prevented eIF2α phosphorylation without affecting PERK activation, suggesting that PC2 facilitates PERK-induced phosphorylation of eIF2α. Pathogenic mutants of PC2 were not able to repress cell proliferation (46). It is of interest that polycystic kidneys also were noted to develop in aquaporin-11 (AQP11)-null mice, in which proliferation of tubular cells with ER vacuolization was seen. This was followed by upregulation of ER stress markers and tubular cell apoptosis, with ultimate development of renal failure (73). Thus an emerging role exists for ER stress in the pathogenesis of PKD.

Primary Glomerular Disease

Nonproliferative glomerulonephritis

Minimal-change disease (MCD) is characterized by an absence of pathologic changes on light microscopy, but significant proteinuria develops because of a defect in the glomerular capillary wall, characterized by podocyte foot process fusion. In the rodent puromycin aminonucleoside (PAN) model of MCD, ER stress, as shown by GRP78 upregulation, was seen in podocytes at days 4 and 5, coinciding with the development of heavy proteinuria. This was associated with a change in the cellular localization of nephrin, from predominantly plasma membrane to cytoplasm (65). It was further demonstrated that mizoribine, a purine nucleotide biosynthesis inhibitor with immunosuppressant properties, known to reduce proteinuria in PAN nephrosis (88), normalized the abnormal processing and localization of nephrin induced by ER stress *in vitro* (65).

Glucocorticoids, commonly used to treat minimal-change disease and several other proteinuric disorders, also were demonstrated to protect against ER stress–induced improper cellular localization of nephrin by stimulating ATP production (17). In this model, glucose starvation led to ATP depletion and ER stress in HEK293 cells, and both dexamethasone and mizoribine were thought to function at least partly through restoration of ATP, which is essential for proper protein folding (17, 65).

Membranous nephropathy, also a common cause of primary nephrotic syndrome, is characterized by glomerular basement membrane immune deposits. In the corresponding rat model, passive Heymann nephritis (PHN), these immune deposits activate complement, leading to the formation of the membrane attack complex C5b-9 and complement-mediated podocyte injury (67). It has been shown that C5b-9 upregulates GRP78 and GRP94 transcript and protein expression in glomerular epithelial cells in culture, whereas GRP78 downregulation enhanced complement-mediated injury. *In vivo*, both proteins were upregulated in glomeruli in rats with PHN. ER stress preconditioning 4 days before PHN induction with tunicamycin (1 mg/kg IP) or doxorubicin (Adriamycin; 6 mg/kg IV) reduced proteinuria in this model, suggesting a protective role for ER stress protein induction against complement-mediated injury (9). Furthermore, complement-induced reduction of protein synthesis through PERK-dependent eIF2α phosphorylation in glomerular epithelial cells was suggested to serve as a mechanism limiting injury. Supportive of this, enhanced glomerular phosphorylation of eIF2α was observed in glomeruli of PHN rats, and fibroblasts from PERK-null mice showed increased susceptibility to complement-mediated toxicity (8).

Several studies assessed the presence of ER stress markers in human kidney biopsies. In MCD, transcript upregulation of *GRP78*, oxygen-regulated protein 150 *(ORP150)*/*HYOU1*, and XBP1 was seen, with increased protein expression of ORP150/HYOU1, but not GRP78, with immunofluorescence (48). Various proteinuric conditions, including MCD, membranous nephropathy, and focal segmental glomerulosclerosis (FSGS) were associated with increased podocyte CHOP/GADD153 expression (3), and upregulation of GRP78 also was seen in membranous nephropathy and FSGS (59).

Proliferative glomerulonephritis

Proliferative glomerulonephritis refers to a group of glomerular diseases of various etiologies characterized by proliferation of mesangial cells, podocytes, parietal epithelial cells, or a combination of these. In biopsies from patients with proliferative glomerulonephritis [membranoproliferative glomerulonephritis (MPGN) and rapidly progressive glomerulonephritis (RPGN)], both CHOP/GADD153 and GRP78 were more abundantly expressed than in nonproliferative glomerular conditions (59), suggesting a more prominent role for ER stress in proliferative renal disease. In the anti-Thy1 rat model of MPGN, GRP78 and ORP150 were shown to be upregulated in glomerular epithelial cells and mesangial cells. When treated 4 days before disease induction with subnephritogenic doses of ER stress inducers tunicamycin (0.3 mg/kg IP) or thapsigargin (0.2 mg/kg IP), histologic damage and proteinuria were reduced (30).

Proteinuria

Proteinuria is a common manifestation of renal disease of diverse etiologies. A common cause of proteinuria is an abnormality in glomerular epithelial cell (podocyte) structure characterized by foot process fusion with resultant disruption of the filtration barrier (slit diaphragm) to proteins (63). In addition to the discussion of ER stress in podocytes in renal diseases associated with heavy proteinuria (see earlier), proteinuria also was associated with an increased risk of progression of renal insufficiency, and as such, the effects of protein on renal tubular cells have been studied.

Exposure of tubular epithelial cells to albumin as an *in vitro* model of proteinuria leads both to activation of profibrogenic mechanisms and to tubular cell injury (71, 103). In immortalized rat proximal tubular cells, albumin-induced apoptosis was accompanied by GRP78 and ORP150 induction, indicative of an ER stress response. Both were also upregulated and associated with apoptosis in proximal tubular cells *in vivo* in PAN-induced proteinuria, indicative of an ER stress response to an increase in filtered proteins (71). Mice in which GRP78/BiP was mutated to remove its ER retention sequence were also more sensitive to bovine serum albumin (BSA)-overload proteinuria, showing significantly greater tubular damage and worse renal function compared with wild-type controls with similar levels of induced proteinuria. This was

associated with increased tubular caspase-12 activation and tubular cell apoptosis (37). These findings thus suggest that ER stress may be induced by proteinuria in the tubular epithelium and may contribute to tubulointerstitial injury associated with chronic proteinuria.

Tubular Injury

Ischemia/reperfusion

Although renal ischemia/reperfusion (I/R) causes acute kidney injury, it also may lead to chronic renal insufficiency if damage to the nephron complement is sufficient. I/R injury has been shown to induce ER stress in the kidney, including induction of XBP-1 splicing followed by upregulation of GRP78, predominantly in the proximal tubule epithelium (82). Interestingly, pretreatment with the UPR inducers thapsigargin (1 mg/kg IP) or tunicamycin (1.5 mg/kg IP) 24 or 48 h before I/R injury respectively, shown to cause GRP78 upregulation at the time of ischemia, both significantly improved renal dysfunction after injury (82). In further work, pharmacologic treatment with 1-(3,4-dihydroxyphenyl)-2-thiocyanate-ethanone, referred to as BiP inducer X (BIX), which selectively upregulates GRP78/BiP without inducing the proapoptotic UPR gene *CHOP/GADD153* (40), protected against I/R injury. Addition of tunicamycin conferred no added protection (81). These studies demonstrate an increase in ER stress in I/R injury.

However, a reduction in ER stress was also suggested in an acute hypobaric model of renal hypoxia, in which downregulation of ER protein-folding chaperones GRP78, GRP94, and calnexin and reduced Xbp1 mRNA splicing were observed (35). The mechanism leading to this effect is unknown, but may reflect a reduced renal requirement for protein synthesis under these conditions. Consistent with these findings, reducing renal protein synthesis through PERK-dependent eIF2α phosphorylation provided a cytoprotective effect in glomerular epithelial cells and limited I/R-dependent glomerular epithelial cell injury (8). This reduction in general protein synthesis, however, excludes UPR-induced genes such as *GRP78*, as well as nephrin, which constitutes a key protein in the maintenance of the podocyte permeability barrier, as discussed earlier (8).

Bax inhibitor-1 (BI-1) also has been shown to have a cytoprotective effect against renal I/R injury associated with modulation of ER stress. I/R injury and renal dysfunction was greater in BI-1–knockout mice in comparison to wild-type animals. This coincided with elevated renal expression of CHOP/GADD153, spliced XBP-1, and nuclear ATF6, indicative of sensitization of the kidneys to ER stress in BI-1–knockout mice (1). These studies in aggregate suggest that modulation of ER stress through pharmacologic intervention to augment the cytoprotective aspects of the UPR (GRP78 upregulation and eIF2α phosphorylation), while downregulating the proapoptotic effects of UPR induction, such as CHOP/GADD153 upregulation, may improve I/R-related renal injury.

Drugs

Evidence for ER stress in the pathogenesis of drug-induced nephrotoxicity is accumulating, with both *in vitro* and *in vivo* evidence emerging.

NSAIDS and prostaglandins

The kidneys produce and secrete prostaglandins that act locally in the regulation of renal function in an autocrine or a paracrine fashion to buffer excessive renal functional changes during physiologic stress (23). It has been shown that blockade of prostaglandin synthesis by the NSAID indomethacin induces ER stress in murine podocytes (38). Conversely, prostaglandin synthesis may be upregulated in response to ER stress, since in cancer cells cyclooxygenase-2 (COX-2) protein was induced by ER-stress agents (29). COX-2 upregulation has been observed in several models of renal injury, and its overexpression in podocytes exacerbated doxorubicin-induced renal toxicity (7).

Reduced prostaglandin synthesis in chronic kidney disease may also augment the cytotoxic effects of ER stress. In a small cohort of patients with chronic kidney disease, combination therapy with angiotensin-converting enzyme (ACE) inhibitor and prostaglandin (26 patients) was shown to reduce the risk of decline in renal function compared with ACE-inhibitor therapy alone (26 patients) by 54% (66). The prostaglandin PGE_2 is produced in mammalian kidneys (33) and 11-deoxy-16,16-dimethyl PGE2 (DDM-PGE2), a stable synthetic analogue of PGE2, was shown to have protective effects on carbon tetrachloride–induced renal damage (85). The mechanism of cytoprotection by DDM-PGE2 may involve the upregulation of GRP78 protein expression. In LLC-PK1 renal epithelial cells, GRP78 upregulation protected cells from the oxidants (and ER stress inducers) hydrogen peroxide, iodoacetamine, and 2,3,5-*tris*(glutathione-*S*-yl) hydroquinone, a nephrotoxic metabolite of hydroquinone (33, 93). The synthesis of specific prostaglandins in the kidney may therefore buffer the cytotoxic effects of ER stress inducers and play a role in preventing the progression of chronic kidney disease.

Acetaminophen

Acetaminophen has been associated with acute renal failure, most commonly in the setting of overdose. This is usually due to tubular necrosis, although associated endothelial injury has also been observed (4). Acetaminophen has been demonstrated to induce ER stress–mediated apoptotic cell death in proximal tubular epithelial cells. ER stress induction was characterized by CHOP/GADD153 upregulation and translocation to the nucleus, as well as by caspase-12 cleavage (55). A metabolite of acetaminophen, *p*-aminophenol (pAP) also was shown to induce ER stress both *in vitro* in renal tubular cells and *in vivo*. In pAP-induced nephrotoxicity in rats, tubular ER stress was seen, characterized by upregulation of XBP1, GRP94, and GRP78 and caspase-12 cleavage (80). Although ER stress preconditioning was protective against pAP toxicity in multiple renal cells, this strategy was not protective *in vivo*. Treatment with a selective calpain inhibitor, however, was effective in reducing renal injury (78, 79). Although previous reports showed that calpain inhibition can prevent induction of GRP78 and ER stress–induced renal cell death (64), pAP-induced XBP1 activation and GRP94 induction were unaltered by calpain inhibition. The mechanism of protection did, however, involve reduction in caspase-12 activation, suggesting an effect on modulation of ER stress responses (79).

Antibiotics

Members of the aminoglycoside class of antimicrobials are well-known causes of acute kidney injury, characterized by tubular injury and necrosis. This occurs as a result of their preferential accumulation in proximal tubular cells (16). Normal rat kidney (NRK) cells treated with geneticin, similar in structure to the clinically commonly used gentamicin, induced apoptosis through both the mitochondrial release of cytochrome *c* as well as through induction of ER stress, the latter associated with m-calpain and procaspase-12 cleavage (34). A role for ER stress was further demonstrated *in vivo*. Increased ER stress markers XBP1, GRP94, and GRP78 were observed in kidney tubules of rats treated with gentamicin (80). This group further demonstrated that ER stress preconditioning of renal tubular cell lines from multiple species was protective against toxicity induced by various drugs, including gentamicin, cyclosporine, and cisplatin (see also later) (78).

Chemotherapeutics

Cisplatin is a widely used chemotherapeutic agent to treat a variety of malignancies. One of its limitations is renal toxicity, characterized by proximal tubular cell dysfunction associated with cell necrosis and apoptosis (12). It was suggested that ER stress plays a role in cisplatin-associated tubular cell toxicity. In LLC-PK1 cells, cisplatin led to cleavage of the ER-specific procaspase-12, and a caspase-12–blocking antibody reduced apoptosis (51). ER stress also was observed *in vivo* in tubular cells in rats administered cisplatin, with increased XBP1 signaling, upregulation of GRP94 and GRP78, and procaspase-12 cleavage (80). In several different cultured tubular cell lines, ER stress preconditioning reduced cisplatin-induced cell toxicity, suggesting a potential for mitigating the toxic clinical effects of this agent (78). Of interest, although cisplatin is characteristically thought to incur cell toxicity through DNA damage, it was recently shown to induce cell death by an alternate mechanism in mouse kidney proximal tubular cells. Here, cytoplasmic cyclin-dependent kinase 2 (cdk2), localized to the ER/Golgi compartments, was a mediator of cisplatin toxicity, and cdk2 inhibition prevented cisplatin-induced and ER stress (tunicamycin or thapsigargin)–induced apoptosis (102).

Heavy metal–induced injury

Heavy metals such as cadmium, mercury, and lead can accumulate in the renal tubular epithelium and lead to renal tubular injury (2). *In vitro*, it has been shown that these agents induce ER stress in renal tubular epithelial cells. In the rat renal proximal tubular cell line NRK52E, cadmium, nickel, and cobalt induced ER stress characterized by GRP78 and CHOP/GADD153 upregulation (26). In LLC-PK1 cells, cadmium chloride (10–50 μM)-induced ER stress led to apoptosis through induction of CHOP/GADD153, Jun N-terminal kinase (JNK) phosphorylation, and activation of the ATF6 and IRE1 pathways of the UPR (49, 99). *In vivo*, cadmium chloride (12 mg/kg body weight) also was shown to induce ER stress in the murine renal cortex (99). Of interest, cadmium chloride–induced apoptosis of LLC-PK1 cells could be suppressed by overexpressing prosurvival ER stress–response proteins GRP78 and ORP150 (99). This suggests that ER stress is a contributing factor to cadmium-induced nephrotoxicity and that manipulation of the UPR response toward prosurvival gene expression may reduce renal damage. Other heavy metals, including mercury and lead, also were shown to induce GRP78 expression in LLC-PK1 cells (99). Furthermore, although glomerular injury is a later event in heavy metal nephrotoxicity, ER stress (GRP78 upregulation) has also been demonstrated in mesangial cells in response to mercury or lead. Of interest, GRP78 upregulation in response to these heavy metals occurred by a different, as yet uncharacterized, mechanism from thapsigargin inducibility (15).

Calcineurin inhibitors

The calcineurin inhibitors cyclosporine and tacrolimus are commonly used immunosuppressants in renal transplantation. However, nephrotoxicity associated with their prolonged use has been a major contributor to long-term renal allograft dysfunction. Studies primarily focusing on cyclosporine have implicated ER stress in calcineurin-induced nephrotoxicity. Cyclosporine has been shown to induce ER stress and apoptosis in renal tubular epithelial cells *in vitro* (75). *In vivo*, in a rat model of chronic cyclosporine-induced nephropathy, early (7 days) responses to cyclosporine treatment activated both prosurvival aspects of the UPR, including GRP78 induction, as well as proapoptotic responses including caspase-12 and CHOP/GADD153 upregulation. Longer-term treatment (28 days) resulted in decreased GRP78 expression, further increased CHOP/GADD153 expression, and apoptotic cell death, suggesting that cyclosporine induces nephropathy through an ER stress–mediated pathway (22). Further, in kidney transplant biopsies cyclosporine treatment was associated with upregulation of GRP78 as compared with normal kidney, suggesting a role for ER stress in cyclosporine-induced transplant nephrotoxicity (76). Of interest, comparison of the effects of cyclosporine and tacrolimus revealed that ER stress induction in endothelium seemed to be specific to cyclosporine, with GRP78 upregulation, cell death, and CHOP/GADD153 expression observed only with cyclosporine treatment (5). Thus, although associated with similar nephrotoxic effects, these two calcineurin inhibitors may induce toxicity through different mechanisms.

Kidney transplant

When the total number of functional nephrons is reduced, as occurs in uninephrectomy, renal protein synthesis is increased. In rats 24 h after uninephrectomy, protein synthesis in proximal renal tubules, as measured by ^{14}C-valine incorporation, was approximately doubled. Without a concomitant increase in protein degradation, this leads to renal hypertrophy and maintenance of the overall glomerular filtration rate (87). ER stress induction inhibits general protein synthesis through PERK-induced eIF2α phosphorylation, preventing mRNA translation through inhibition of the cap-dependent mechanism of translation initiation (36). This effect would counter the necessary hypertrophy required to maintain renal function in transplanted kidneys and may contribute to allograft dysfunction. Indeed, in protocol biopsies from unselected renal transplant recipients at 3 and 12 months after transplant, GRP78 was upregulated in tubular cells in transplanted as compared with nontransplanted kidneys. This was found irrespective of whether antirejection treatment in-

cluded the calcineurin inhibitor cyclosporine, although upregulation was seen to a greater extent in those using cyclosporine as compared with sirolimus or azathioprine (76). The use of protocol biopsies in this study should remove the possibility of sampling bias, as might occur if only patients with graft failure were studied. Further, it was shown that the graft weight/recipient weight ratio is a significant determinant of longer-term renal function and proteinuria in live donor kidney recipients who have good immediate post-transplant renal function and have not had significant ischemic injury or episodes of rejection (69). An interesting hypothesis would be that decreased metabolic demand on the graft, and thus less demand for protein synthesis, may improve overall functional outcome. However, the role of the ER stress/UPR induction in graft survival and long-term function is an area that is in need of further research.

Crystal-induced injury

Calcium oxalate monohydrate is the most common constituent of kidney stones. *In vitro*, calcium oxalate monohydrate adheres to renal tubular epithelial cells, inducing cellular injury leading to apoptosis (90, 95). *In vivo*, increased calcium oxalate urinary excretion is also associated with apoptosis of distal tubular epithelial cells (62). A role for ER stress has been implicated in one study in tubular cell responses to these crystals. Here, Madin-Darby canine kidney (MDCK) distal renal tubular cells exposed to high-dose (1 mg/ml) calcium oxalate monohydrate crystals showed upregulation of the ER chaperones GRP78 and ORP150 (90). This likely represents a protective cellular response, although not sufficient to prevent cell death.

Diabetic Nephropathy

Although ER stress has been implicated in the pathophysiology of diabetes as well as its complications, including retinopathy and cardiovascular disease (14, 20, 74), only recently has attention turned to a potential role for ER stress in the pathogenesis of diabetic nephropathy. In type 1 diabetes induced by streptozotocin, GRP78 and CHOP/GADD153 were upregulated in whole kidney after 4 months of diabetes. This was associated with increased TUNEL positivity and caspase-12 activation, associating ER stress and apoptotic cell death with diabetic nephropathy (50). A role for increased apoptosis has been well documented in glomeruli and tubules in both animal models and patients with diabetic nephropathy (41, 42, 77, 96). Microarray analysis also identified upregulation of *XBP1* and ER chaperones (*HSPA5/GRP78, ORP150/HYOU1*) in kidney biopsies from patients with established diabetic nephropathy. Of interest, ER stress was not observed in biopsies from patients with less-advanced diabetic nephropathy (*i.e.*, creatinine $<125\,\mu M$), suggesting a correlation between degree of renal injury and ER stress induction (48). *In vitro*, longer-term (6 days) glucose exposure also led to upregulation of these genes (*XBP1, GRP78, ORP150/HYOU1*) in tubular epithelial cells, an effect that was enhanced by coadministration of albumin to mimic proteinuria (48).

Advanced glycation end products (AGEs), thought to be involved in the pathogenesis of diabetic nephropathy (18), have also been shown to induce ER stress. In murine podocytes, AGEs increased apoptosis as well as GRP78 expression. Treatment with the low-molecular-weight protein-folding chaperone tauroursodeoxycholic acid (TUDCA) inhibited both GRP78 upregulation and apoptosis in these cells, suggesting a potential novel treatment strategy for diabetic nephropathy (6).

Angiotensin II

Angiotensin II is a well-established mediator of the progression of renal disease of diverse etiologies, with blockade of angiotensin II action associated with improved clinical outcome in proteinuric renal disease (43). The renoprotective effects of angiotensin II blockade, independent of blood-pressure reduction, are complex and not fully understood, but it is of interest that angiotensin II has been demonstrated to induce ER stress in other cells. In cardiac myocytes, angiotensin II (10^{-9} *M*) upregulated ER chaperones and CHOP/GADD153 and induced apoptosis (72). Angiotensin II–receptor inhibition prevented ER stress and apoptosis in heart failure induced by transverse aortic constriction (72). Whether the renoprotective effect of angiotensin II blockade in chronic kidney disease involves inhibition of angiotensin II–induced ER stress remains to be examined.

Relation between ER stress and oxidative stress

The generation of reactive oxygen species (ROS) has been implicated in the pathogenesis of most acquired renal diseases. There is also evidence that ROS may be involved in ER stress–induced cytotoxicity. The strong oxidant peroxynitrite induced ER stress in vascular endothelial cells, characterized by upregulation of GRP78, GRP94, and TDAG51, as well as eIF2α phosphorylation and apoptosis (10). In tubular epithelial LLC-PK1 cells, both peroxynitrite and superoxide led to ER stress, associated with GRP78 and CHOP/GADD153 induction and apoptosis (100). Furthermore, ROS may mediate ER stress caused by various pathologic agents. For example, the antioxidant *N*-acetylcysteine (1 m*M*) or overexpression of manganese superoxide dismutase significantly decreased ER stress and apoptosis induced by the heavy metal cadmium chloride in LLC-PK1 cells, suggesting that superoxide was involved in cadmium-induced ER stress (100). *In vivo*, cisplatin-induced nephrotoxicity previously shown to involve ER stress was independently seen to induce glutathione depletion in rat kidneys, with nephrotoxicity being partly ameliorated by *N*-acetylcysteine (56, 80).

Conversely, ER stress may also lead to oxidative stress. ROS generation was observed in a model of ER stress induction resulting from the misfolding and aggregation in the ER of coagulation factor VIII. This was associated with CHOP/GADD153-mediated apoptosis. Of interest, antioxidant treatment reduced UPR activation and apoptosis and improved the secretion of the misfolded protein coagulation factor VIII (58).

ER stress preconditioning has also been shown to alleviate the cytotoxic effects of ROS. In LLC-PK1 cells, cytotoxicity caused by hydrogen peroxide was inhibited by ER stress preconditioning with various agents. This pretreatment elevated GRP78 and inhibited the cytosolic Ca^{2+} elevations induced by hydrogen peroxide (28). Subsequent work demonstrated that GRP78 overexpression reduces the increase in cytosolic Ca^{2+} caused by ER Ca^{2+} releasers (11), suggesting that ER stress preconditioning may protect against a broad array of oxidant-induced apoptosis by

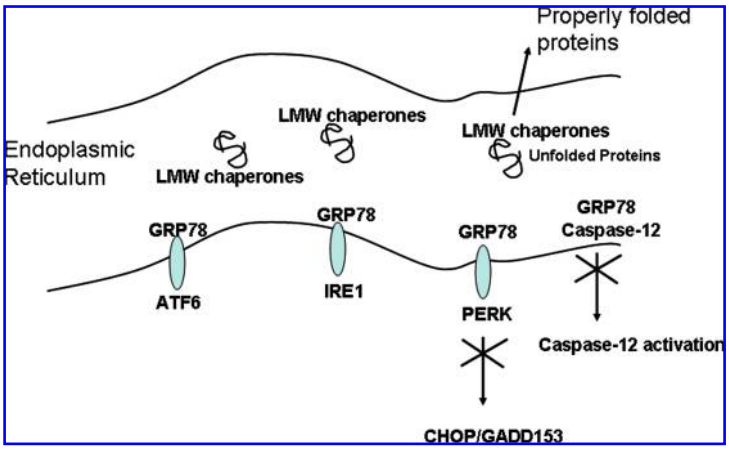

FIG. 3. Potential therapeutic interventions aimed at preventing renal disease through maintenance of ER homeostasis. Inhibition of the unfolded protein response (UPR) by increasing the protein-folding capacity of the ER by treatment with low-molecular-weight protein-folding chaperones (LMW chaperones), such as tauroursodeoxycholic acid (TUDCA) or 4-phenylbutyric acid (4-PBA) may hold promise in alleviating the progressive decline in renal function associated with chronic kidney disease. These interventions may function by inhibiting the induction of proapoptotic ER stress-response genes such as *CHOP/GADD153* and caspase-12 activation. GRP78 remains unassociated with misfolded proteins, also inhibiting the proapoptotic response. LMW chaperones may also rescue the trafficking defect, likely through an increase in proper folding, in the ER, of proteins such as nephrin and polycystin-2, allowing them to localize properly to the membrane and to restore their function.

limiting oxidant-induced cytoplasmic Ca^{2+} overload. Likewise, in LLC-PK1 renal epithelial cells, iodoacetamide cytotoxicity was prevented by increased expression of the ER Ca^{2+}-binding proteins GRP78 and calreticulin *via* blocking cytosolic Ca^{2+} increase and ROS generation (52). Thus, in aggregate studies suggest a bidirectional relation between ER stress and oxidative stress. The effectiveness of antioxidants in alleviating the severity of ER stress and UPR-mediated cell death deserves further study.

Potential therapeutic strategies to reduce ER stress–induced renal pathology

Increasing evidence supports a role for ER stress in the pathogenesis of a wide range of renal diseases. This thus presents an opportunity for the development of novel therapeutic approaches to prevent and treat various nephropathies (Fig. 3). In congenital diseases, a role for protein misfolding and hence mislocalization associated with at least some of the disease-causing mutations in proteins, such as nephrin, podocin, and polycystins, supports investigation of chemical chaperones such as 4-PBA or TUDCA to correct the abnormal protein localization (54, 70). However, although at least some aspects of protein (nephrin) function were shown to be rescued (54), further studies are required to assess whether this approach can fully rescue protein function and thus prevent renal disease progression. It is likely that genetic screening would be required to identify those individuals with mutations amenable to therapy.

Small-molecule chemical chaperones may be beneficial in the treatment of a wider range of renal diseases. For example, abnormalities in the trafficking and localization of slit diaphragm proteins such as nephrin have also been found in noninherited proteinuric renal disease (65). It was reported that glucocorticoids, a widely used treatment for several proteinuric renal diseases, protect against ER stress–induced improper cellular localization of nephrin (17). In cell culture studies, encouraging results with small-molecule chaperones have also been observed in studies related to diabetic nephropathy (6).

Cell preconditioning with ER stress has been examined as a potential protective maneuver against induction of renal injury. *In vitro*, this approach has been effective in tubular cells in response to numerous agents including gentamicin, cyclosporine, cisplatin, and *p*-aminophenol (78). This did not necessarily translate to protection *in vivo*, as was found with *p*-aminophenol, demonstrating the need for *in vivo* correlation of *in vitro* studies (79). In the Heymann nephritis and Thy 1 models of membranous nephropathy and MPGN, respectively, ER stress preconditioning did reduce proteinuria and renal injury (9, 30). However, membranous nephropathy is a chronic condition, and it is possible that the clinical applicability for treatment with such a strategy would be restricted to those agents and conditions that cause acute nephrotoxicity, such as I/R injury (82).

Finally, several other interesting approaches to mitigating the cytotoxic effects of ER stress have been studied and await further investigation. Treatment with a calpain inhibitor was shown to be effective in reducing renal injury in *p*-aminophenol tubular toxicity (78, 79). Whether calpain inhibition functions through modulation of ER stress or another pathway is not clear and may be disease specific (79). Selectively upregulating GRP78 with BIX, which does not induce the proapoptotic aspects of the UPR response, is another possible approach to be tested (40, 81). Finally, the use of specific prostaglandins or their analogues, such as DDM-PGE_2, may ameliorate ER stress and associated renal cell injury (33). Thus, although we are still at the early stages of potential therapeutic development, alteration of ER stress has emerged as a promising and novel therapeutic approach to renal disease of diverse etiologies.

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Abbreviations Used

ATF4 = activated transcription factor 4
ATF6 = activating transcription factor 6
ATP = adenosine triphosphate
Bcl-2 = B-cell leukemia/lymphoma 2
CHOP = C/EBP homologous protein
DMSO = dimethyl sulfoxide
eIF2α = eukaryotic translation initiation factor 2α
ER = endoplasmic reticulum
GADD153 = growth arrest and DNA-damage–inducible protein 153
GRP78 = glucose–regulated protein 78-kDa
GRP94 = glucose-regulated protein 94-kDa
IRE1 = inositol-requiring enzyme 1
JNK = Jun N-terminal kinase
ORP150 = oxygen-regulated protein
4-PBA = 4-Phenylbutyrate
PDI = protein disulfide isomerase
PERK = PKR-like ER kinase
PGE_2 = prostaglandin E_2
PKR = double-stranded RNA-dependent protein kinase
TDAG51 = T-cell death–associated gene 51
TUDCA = tauroursodeoxycholic acid
TUNEL = terminal deoxynucleotidyl transferase biotin dUTP nick-end labeling
UPR = unfolded protein response
XBP-1 = X-box–binding protein 1

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