Endoplasmic reticulum stress in proteinuric kidney disease

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Endoplasmic reticulum (ER) stress refers to physiological or pathological states that result in accumulation of misfolded proteins in the ER. To handle misfolded proteins, the ER has in place quality control mechanisms, including the unfolded protein response and ER-associated degradation (ERAD). ER stress in renal pathophysiology is a relatively new area of research. Mice heterozygous for a mutation in the ER chaperone, BiP, develop glomerulosclerosis and tubulointerstitial disease. Induction of ER stress in glomerular cells has been described in experimental models of membranous nephropathy and membranoproliferative glomerulonephritis, and exogenous induction of ER stress ('preconditioning') reduced proteinuria. In human kidney biopsies, markers of ER stress in glomeruli have been identified in various noninflammatory and inflammatory glomerulopathies. A tubulointerstitial ER stress response, in some cases associated with tubular cell apoptosis, may occur in glomerular diseases associated with proteinuria, including puromycin aminonucleoside nephrosis, protein overload, and experimental and human diabetic nephropathy. Certain missense mutations in nephrin and podocin, as well as underglycosylation of nephrin, result in misfolding and retention in the ER, and eventually ERAD. Understanding the various aspects of ER stress will provide an opportunity for development of novel therapeutic strategies for proteinuric diseases.

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The endoplasmic reticulum (ER) is a membranous network that extends throughout the cytoplasm of a cell, and is contiguous with the nuclear envelope. The ER serves as a site for the folding, assembly, and degradation of proteins, as well as for synthesis of steroids, cholesterol, and other lipids. In addition, the ER is a major intracellular storage site for calcium. Secretory, lumenal, and membrane proteins are translocated into the lumen of the ER shortly after initiation of synthesis (Figure 1).[1] These proteins are covalently modified and attain their correctly folded conformation in the ER through ER-resident enzymes and chaperones. Protein folding is catalyzed by peptidyl prolyl isomerases, whereas glycosylation involves glycosidases and mannosidases. Folding-competent states are maintained by classical chaperones, for example, the glucose-regulated proteins (GRP)94 and BiP (GRP78), and lectin-like chaperones, for example, calnexin and calreticulin. ER stress refers to physiological or pathological states, which may increase the demand for protein folding, or disrupt the processes by which proteins fold, resulting in an accumulation of misfolded proteins in the ER lumen. To rescue misfolded proteins, the ER has in place quality control mechanism, including the unfolded protein response (UPR)[1–5] and ER-associated degradation (ERAD).[1,6–8]

UPR

The UPR is a coordinated stress response that upregulates the capacity of the ER to process abnormal proteins (Figure 1).[2–4] The major UPR signaling pathways are initiated by three protein sensors, activating transcription factor-6 (ATF6), inositol requiring-1α (IRE1), and PERK (PKR-like ER kinase). In resting cells, the three sensors are in an inactive state, by association with the ER chaperone, BiP. Upon accumulation of misfolded proteins in the ER, or depletion of ER calcium stores, ATF6 is released from BiP and moves to the Golgi, where it is cleaved by site-1 and site-2 proteases. The cleaved cytosolic fragment, which has a DNA-binding domain (containing the basic leucine zipper motif and a transcriptional activation domain), migrates to the nucleus to activate transcription of ER chaperones and enzymes that promote protein folding, maturation, secretion, and ERAD. In parallel with ATF6, IRE1 autophosphorylates and activates its endoribonuclease activity, cleaving X-box-binding protein-1 (XBP1) mRNA and changing the reading frame

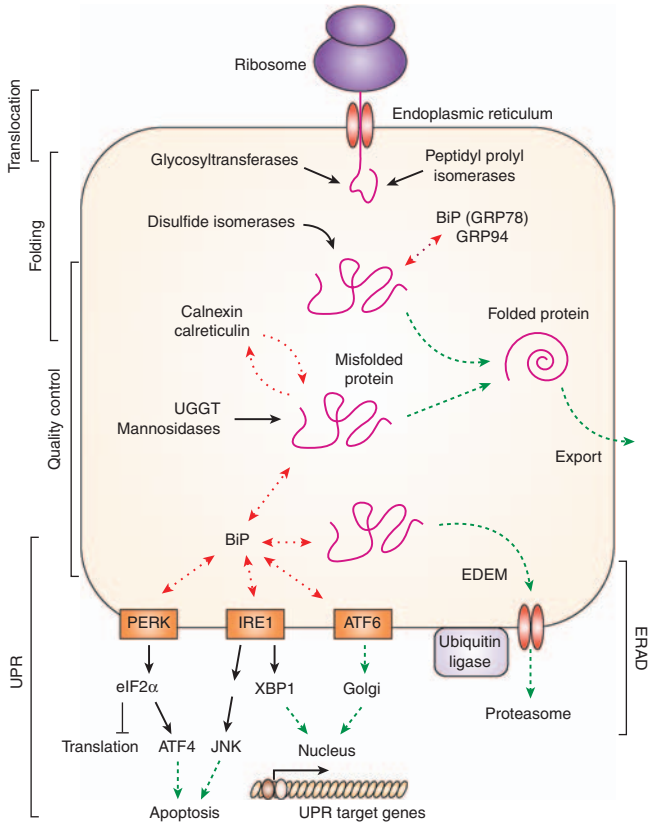

Figure 1 | Protein folding, quality control, and signaling in the endoplasmic reticulum (ER). Solid arrow indicates enzymatic catalysis, dotted arrow indicates an interaction, dashed arrow indicates transport. ATF4, activating transcription factor-4; ATF6, activating transcription factor-6; eIF2α, eukaryotic translation initiation factor-2α subunit; EDEM, endoplasmic reticulum degradation-enhancing α-mannosidase-like protein; ERAD, endoplasmic reticulum-associated degradation; GRP94, glucose-regulated protein 94; IRE1, inositol requiring-1α; JNK, c-Jun N-terminal kinase; PERK, PKR-like ER kinase; UGGT, UDP-glucose:glycoprotein glucosyltransferase; UPR, unfolded protein response; XBP1, X-box-binding protein-1. Adapted from Chevet *et al.*[1]

to yield a potent transcriptional activator. XBP1 functions in parallel with ATF6 to activate transcription of the aforementioned genes. A third aspect of the UPR involves PERK, which is activated through homodimerization and trans-phosphorylation, allowing PERK to phosphorylate the eukaryotic translation initiation factor-2α subunit (eIF2α). This process reduces initiation AUG codon recognition; thus, the general rate of translation is reduced, which aims at decreasing the protein load on a damaged ER. However, selective mRNAs, typically those that contain short open reading frames in the 5′-untranslated region, can be preferentially translated under these conditions. Such mRNAs include ATF4, a transcription factor that can induce expression of UPR target genes. Although BiP may serve as a master regulator of the UPR sensors (ATF6, IRE1, and PERK), more recent studies suggest that IRE1 (and possibly PERK) may bind misfolded proteins directly.[4] Such direct recognition may allow for a more nuanced set of responses.

QUALITY CONTROL AND ERAD

Calnexin and calreticulin are ER chaperones involved in the folding of glycoproteins.[1,7] ER quality control is mediated through recognition of glycan moieties bound to proteins. Unfolded or misfolded proteins are reglucosylated by UDP-glucose:glycoprotein glucosyltransferase (UGGT), and ER mannosidases affect retention time of misfolded proteins with ER chaperones (Figure 1). Calnexin, UGGT, and mannosidases allow misfolded proteins multiple chances to acquire a correctly folded conformation (calnexin chaperone cycle). However, prolonged retention of misfolded proteins leads to ERAD.[6,8] Mannose trimming of N-linked glycan has an important role in ERAD. ER degradation-enhancing α-mannosidase-like protein (EDEM) functions as the mannose$_8$-binding lectin in the ERAD pathway, and is a key molecule that recognizes misfolded glycoproteins. EDEM helps misfolded proteins leave the calnexin cycle toward degradation. The misfolded proteins are retrotranslocated into the cytosol, where they undergo ubiquitination, a covalent modification that marks the protein for destruction by the proteasome. ER stress may upregulate some of the proteins involved in ERAD, for example, EDEM, which lies downstream of IRE1. The UPR and ERAD are intimately linked; thus, UPR induction may increase ERAD capacity, and loss of ERAD may lead to UPR induction.

CONSEQUENCES OF THE UPR

Activation of the UPR appears to be a generalized process occurring in many cell types. In certain cell types, the UPR may be important for normal physiological function, including those cells with a high rate of protein synthesis or whose primary function is the production of secretory- or membrane-resident proteins. Examples include pancreatic-β cells, which require the PERK/eIF2α pathway for maintenance of function and prevention of cell failure, and plasma cells, where the IRE1/XBP1 pathway is necessary for the production of antibodies.[2] As discussed above, induction of the UPR allows cells to recover from stress, and once activated, the UPR may be protective to additional insults.[3,4] In contrast, substantial/prolonged ER stress may be cytotoxic, and lead to apoptosis (Figure 1).[3,4,9] The proapoptotic effector pathways may include the ATF4-mediated induction of C/EBP homologous protein-10 (CHOP/GADD153), a gene transcribed preferentially after eIF2α is phosphorylated by PERK, and the activation of caspase-12, or activation of apoptosis signal-regulating kinase-1 and c-Jun N-terminal kinase, downstream of IRE1 (Figure 1).

ER STRESS IN RENAL PATHOPHYSIOLOGY

The role of ER stress in renal pathophysiology is a relatively new area of research. A few comprehensive reviews on this subject have appeared recently.[10–13] The present review highlights ER stress in conditions primarily involving the glomerulus, focusing on glomerular pathophysiology and potential consequences of proteinuria on renal tubular cells.