Participation of endoplasmic reticulum stress in the pathogenesis of spontaneous glomerulosclerosis–Role of intra-renal angiotensin system

MOHAMMAD A. AMINZADEH, TADASHI SATO, and NOSRATOLA D. VAZIRI

IRVINE, CALIF; AND SAGA, JAPAN

Endoplasmic reticulum (ER) is the site of synthesis, folding, assembly, and degradation of proteins. Disruption of ER function leads to ER stress, which is marked by accumulation of unfolded proteins in the ER lumen. Detection of unfolded proteins by the ER membrane receptors triggers the "unfolded protein response (UPR)" designed to restore ER function *via* activation of the adaptive/cytoprotective responses. Failure of UPR or persistent stress triggers activation of ER stress-mediated apoptotic pathway. Several *in vivo* and *in vitro* studies have demonstrated the association of ER stress with glomerular diseases. Imai rats develop progressive glomerulosclerosis (GS), which is associated with oxidative stress, inflammation and activation of intra-renal angiotensin system, and can be prevented by AT-1 receptor blockade (ARB). Since persistent oxidative and inflammatory stresses trigger ER stress-induced apoptosis and tissue injury, we hypothesized that kidneys in the Imai rats may exhibit failure of the adaptive and activation of the apoptotic ER stress responses, which could be prevented by ARB. To this end 10-week old Imai rats were randomized to untreated and ARB-treated groups and observed for 24 weeks. At age 34 weeks, untreated rats showed heavy proteinuria, azotemia, advanced GS, impaired ER stress adaptive/cytoprotective responses (depletion of UPR-mediating proteins), and activation of ER stress apoptotic responses. ARB treatment attenuated GS, suppressed intra-renal oxidative stress, restored ER-associated adaptive/cytoprotective system, and prevented the ER stress mediated apoptotic response in this model. Thus, progressive GS in Imai rats is accompanied by activation of ER stress-associated apoptosis, which can be prevented by ARB. (Translational Research 2012;160:309–318)

Abbreviations: ER = endoplasmic reticulum; UPR = unfolded protein response; GS = glomerulosclerosis; AT-1 = angiotensin II receptor type1; ARB = angiotensin receptor-1 blockade; GRP94 = glucose-regulated protein 94; GRP78 = glucose-regulated protein 78, GRP78 also known as BiP; PERK = pancreatic ER kinase (PKR)-like ER kinase; ATF6 = activating transcription factor 6; IRE1 = inositol-requiring enzyme 1; eIF2α = eukaryotic translation initiation factor-2α; XBP1 = X-box-binding protein-1; Ask1 = apoptosis-signal-regulating kinase-1; NFκB = nuclear factor kappa B; Bcl2 = B-cell lymphoma 2 family of proteins; FSGS = focal segmental glomerulosclerosis; Nrf2 = nuclear factor-erythroid-2-related factor 2; BAX = Bcl-2–associated X protein; JNK = c-Jun N-terminal kinase; Keap1 = Kelch-like ECH-associated protein 1; MAPKKK = mitogen

From the Division of Nephrology and Hypertension, University of California Irvine, Irvine, Calif; Department of Pediatrics, NHO Ureshino Medical Center, Saga, Japan.

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Reprint requests: Nosratola D. Vaziri, MD, MACP, University of California, Irvine Medical Center, Division of Nephrology and Hypertension, The City Tower, Suite, 400, Orange, CA 92868; e-mail: ndvaziri@uci.edu.

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activated protein kinase kinase kinase; LC3 = microtubule-associated protein light chain 3; GAPDH = glyceraldehyde 3-phosphate dehydrogenase

AT A GLANCE COMMENTARY

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Background

Progressive glomerulosclerosis (GS) is associated with oxidative stress, inflammation, and activation of intra-renal angiotensin system in Imai rats, and can be prevented by AT-1 receptor blockade (ARB). Since persistent oxidative and inflammatory stresses trigger endoplasmic reticulum (ER) stress-induced apoptosis and tissue injury, we hypothesized that kidneys in the Imai rats may exhibit failure of the adaptive and activation of the apoptotic ER stress responses that could be prevented by ARB.

Translational Significance

Restoration of ER-associated adaptive/cytoprotective system and prevention of ER stress mediated apoptotic responses reveals another salutatory effect of ARB treatment in progressive GS.

The endoplasmic reticulum (ER) consists of a membranous network that is contiguous with the nuclear envelope and extends throughout the cytoplasm. It serves as the principal site of synthesis, folding, assembly, and degradation of secreted, membrane-bound, and certain organelle-targeted proteins, production of steroids, cholesterol, and other lipids and the major intracellular reservoir of calcium. Newly synthesized proteins are released into the lumen of ER wherein ER-resident enzymes and chaperones mediate their covalent modification and correct folded conformation. In the ER lumen, peptidyl-prolylisomerase catalyzes protein folding, glycosidases and mannosidases mediate protein glycosylation and classical chaperones such as glucose-regulated proteins (GRP) 94 and GRP78 (BiP), and lectin-like chaperones, such as calnexin and calreticulin, maintain their proper folding states.

Proper functioning of the ER is critical for the cell function and survival. Conditions that disrupt ER function result in ER stress, which is marked by accumulation and aggregation of unfolded proteins in the ER lumen. Accumulation of the unfolded proteins is detected by ER membrane receptors, which trigger an adaptive/cytoprotective response termed "unfolded protein response (UPR)" to restore normal ER function and cell survival *via* transmission of signals to the nucleus and cytoplasm. The UPR represents a concerted and complex cellular response mediated by 3 ER transmembrane receptors including pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). At resting condition, these ER stress receptors are held in an inactive state by the ER chaperone, GRP78. Accumulation of unfolded proteins triggers the UPR by promoting dissociation and activation of these receptors from GRP78. Once released, ATF6 migrates to the Golgi apparatus where it is activated *via* cleavage by site-1 and site-2 proteases. It then migrates to the nucleus to promote transcription of ER chaperones and enzymes involved in protein folding, maturation, and secretion. Simultaneously, PERK is activated *via* its homodimerization and transphosphorylation. This allows PERK to phosphorylate the eukaryotic translation initiation factor-2α subunit (eIF2α), which by lowering the initiation AUG codon recognition helps to slow the translation rate, thereby reducing the protein load on the damaged ER. Finally, IRE1 undergoes autophosphorylation and activation of its endoribonuclease activity, which by cleaving X-box-binding protein-1 (XBP1) mRNA and changing its reading frame, yields a potent transcriptional activator. Spliced XBP1, in turn, works in parallel with ATF6 to promote gene transcription of ER enzymes and chaperons (Fig 1).

The UPR is an adaptive/cytoprotective response designed to reduce accumulation of unfolded proteins and restore ER function and cell survival. However, failure of UPR and/or persistence of stress trigger the activation of the ER stress-induced apoptotic responses.[1-8] Several apoptotic mediators have been recognized in relation to the ER stress. They include apoptosis-signal-regulating kinase-1 (Ask1), nuclear factor kappa B (NFκB), IRE1, and B-cell lymphoma 2 family of protein (Bcl2) (Fig 1).[9-12]

There is increasing evidence for the role of ER stress in the pathogenesis of diverse illnesses including kidney diseases. ER stress is present in glomerular cells from the animal models of membranous nephropathy and membranoproliferative glomerulonephritis.[13-15] Development of proteinuria in animals with