

# Endoplasmic Reticulum and Its Role in Diabetic Nephropathy

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Endoplasmic reticulum produces folds and exports proteins. There are studies showing the role of endoplasmic reticulum in pathogenesis of diabetic complications including diabetic nephropathy. This article reviews the pathophysiology associated with the role of endoplasmic reticulum in diabetic nephropathy and the therapeutic aspects of this finding.

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## INTRODUCTION

Diabetic nephropathy (DN) is one of the most common microvascular chronic complications associated with diabetes mellitus and is the most common cause of end-stage renal disease.<sup>1</sup> Diabetic nephropathy develops over several years and involves characteristic pathological changes such as the excessive accumulation of extracellular matrix, glomerulosclerosis, tubule dilatation and atrophy, as well as interstitial fibrosis.<sup>2</sup>

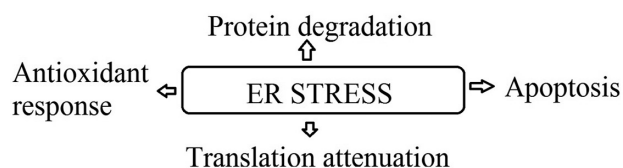
Literature shows the role of endoplasmic reticulum (ER) in the pathogenesis of diabetic complications including diabetic nephropathy. Endoplasmic reticulum is a type of organelle in the cells of eukaryotic organisms that forms an interconnected network of flattened membrane-enclosed sacs or tubes. The functions of the ER can be summarized as the synthesis and export of proteins and membrane lipids. The ER serves many general functions, including the folding of protein molecules in sacs called cisternae and the transport of synthesized proteins in vesicles to the Golgi apparatus. Correct folding of newly made proteins is made possible by several ER chaperone proteins, including protein disulfide isomerase.<sup>3</sup>

## ENDOPLASMIC RETICULUM STRESS

A number of studies have suggested that diabetes mellitus, the hallmark of which consists of elevated plasma glucose, is consistently associated with oxidative stress, a chronic low-grade inflammation and ER stress.<sup>4,5</sup> These processes are ultimately integrated in the pathogenesis of diabetes mellitus.

Endoplasmic reticulum is responsible for protein folding, maturation, quality control, and trafficking. If final tertiary structure cannot be achieved, misfolded proteins are then transported back to the cytosol and subjected to ubiquitination and proteasome-dependent degradation, a process referred to as ER-associated degradation.<sup>6</sup>

When ER becomes stressed due to the accumulation of newly synthesized unfolded proteins, it is referred to as "ER stress," which results in the activation of intracellular signal transduction pathways to restore normal ER function. This activation is called the unfolded protein response (Figure 1).<sup>7</sup> Unfolded protein response is activated to decrease unfolded protein and to increase protein folding capacity.<sup>8</sup> The unfolded protein response is mediated by at least 3 transmembrane proteins, including inositol-requiring enzyme 1, protein-kinase-RNA-like ER kinase, and activating transcription factor 6.<sup>9</sup> Under unstressed conditions, these transmembrane proteins are maintained in an inactive state by binding to the major ER chaperone, glucose-regulated protein 78 or binding immunoglobulin protein, at the side of the ER lumen. During ER



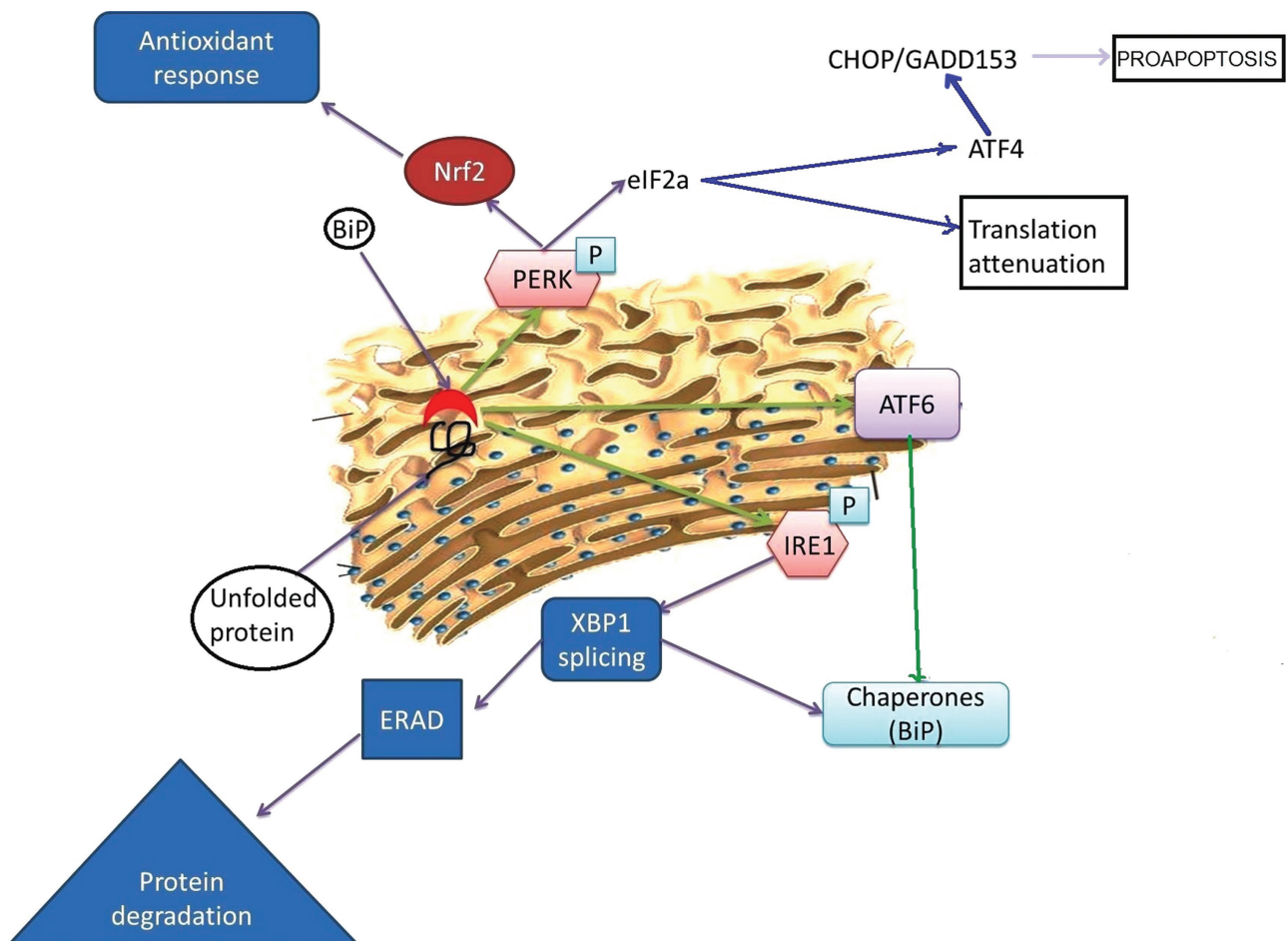
**Figure 1.** Unfolded protein response leads to translation attenuation, protein degradation, antioxidant response, and proapoptosis activity. ER indicates endoplasmic reticulum.

stress, binding immunoglobulin protein is displaced to interact with misfolded luminal proteins, resulting in the release of inositol-requiring enzyme 1, protein-kinase-RNA-like ER kinase, and activating transcription factor 6, and subsequently leading to their activation.<sup>10</sup> Protein-kinase-RNA-like ER kinase activation results in phosphorylation of the  $\alpha$  subunit of eukaryotic translation initiation factor 2, leading to rapid reduction in the initiation of messenger RNA translation, and thus reducing the load of new proteins in the ER. Phosphorylation of eukaryotic translation initiation factor 2 $\alpha$  by protein-kinase-RNA-like ER kinase also allows the translation of activating transcription factor 4 that can induce transcription of genes involved in amino acids synthesis and apoptosis, such as CCAAT/enhancer-binding protein homologous protein (CHOP; Figure 2).<sup>11</sup> When the stress cannot be resolved, the signaling pathways switch from

prosurvival to proapoptosis.<sup>12</sup> Subsequently, CHOP induces other proapoptotic genes, resulting in caspase activation. Caspase is also activated by the inositol-requiring enzyme 1 pathway. Caspase activation is the ultimate step in apoptosis.

Different mechanisms including altered glycosylation, nutrient deprivation, and oxidative stress can interrupt the protein folding process and result in ER stress. A study has demonstrated protection against obesity-induced diabetes mellitus in mice by overexpression of ER chaperones, while knockdown of chaperones was diabetogenic.<sup>13</sup> In addition, treatment with chemical ER chaperones that alleviated obesity-induced ER stress led to improvement in insulin sensitivity.<sup>14</sup>

The functional significance of the unfolded protein response is not yet fully understood, but it is known to contribute to the pathogenesis of many diseases including diabetes mellitus,



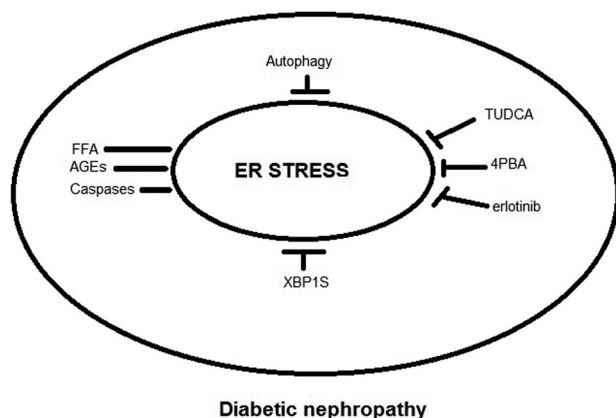
**Figure 2.** Transmembrane proteins and their activation. CHOP indicates CCAAT/enhancer-binding protein homologous protein; Nrf2, nuclear factor (erythroid-derived 2)-like 2; ATF4, activating transcription factor 4; XBP1, X-box binding protein 1; IRE1, inositol-requiring transmembrane kinase/endonuclease-1; ATF6, activating transcription factor 6; and ERAD, endoplasmic reticulum-associated protein degradation.

cancer, atherosclerosis, neurologic diseases, and inflammatory bowel disease.<sup>13</sup>

### ENDOPLASMIC RETICULUM STRESS IN DIABETIC NEPHROPATHY

#### X-Box Binding Protein 1

Accumulating evidence suggests that the ER stress plays an essential role in the development and pathogenesis of diabetic complications.<sup>15</sup> Of note, hyperglycemia induces apoptosis partly through ER stress in the differentiated mouse podocytes, which possibly contributes to the pathogenesis of DN.<sup>16</sup> On the other hand, Lindemeyer and colleagues' microarray mediated study from biopsies of established DN showed that expression of the transcription factor X-box binding protein 1 (XBP1) was increased, also exposure of renal tubular epithelial cells to albumin and high glucose in vitro enhanced expression of genes involved in ER stress.<sup>17</sup> The XBP1 is one of mediators of ER stress and has the capability of inhibiting oxidative stress. Shao and colleagues studied effects of high-glucose milieu on cultured renal mesangial cells (MCs). It was shown that reactive oxygen species production, collagen IV, and fibronectin expressions were increased simultaneously. Upregulating spliced XBP1 (XBP1S) reversed high-glucose-induced reactive oxygen species production and extracellular matrix expressions. On the other hand, knocking-down of intrinsic XBP1S expression induced reverse effects. These results suggested that XBP1S pathway of ER stress was involved in high-glucose-induced oxidative stress and extracellular matrix synthesis (Figure 3).<sup>18</sup>



**Figure 3.** Stimulators and inhibitors of endoplasmic reticulum stress induced by diabetic nephropathy. TUDCA indicates tauroursodeoxycholic acid and FFA, free fatty acid.

#### Free Fatty Acids

Free fatty acids are critically involved in the pathogenesis of type 2 diabetes mellitus. Palmitate could induce podocyte apoptosis via ER stress, which initiates or aggravates proteinuria in DN.<sup>19</sup> On the other hand, Sieber and coworkers showed the monounsaturated palmitoleic and oleic acid could attenuate the palmitic acid-induced cell death. It offers dietary shifting of the free fatty acids balance toward unsaturated free fatty acids that can delay the progression of DN.<sup>20</sup>

#### Advanced Glycation End Products

During a study, advanced glycation end products induced podocyte apoptosis and increased the expression of glucose-regulated protein 78 (an ER stress marker). On the other hand, tauroursodeoxycholic acid (TUDCA) was capable of abolishing changes related to advanced glycation end products. Hence, it was shown that ER stress played an important role in apoptosis induced by advanced glycation end products and that TUDCA could prevent it.<sup>21</sup>

#### Autophagy

Podocytes had a high basal level of autophagy. However, under diabetic condition, defective autophagy facilitated the podocyte injury. The result that TUDCA could restore defective autophagy further indicated that the evolution of autophagy may be mediated by the changes of cytoprotective output in the ER stress.<sup>22</sup>

#### Angiotensin II Receptor Pathway

The kidney of diabetic mice were found to have increased protein expressions of glucose-regulated protein 78 and ER-associated proteins in comparison to normal mice. Lakshmanan and colleagues showed that olmesartan treatment significantly blunted them. Furthermore, the kidney of diabetic mice were found to have significant increment in renal apoptosis which were also attenuated by the olmesartan treatment. Considering all the findings, it is suggested that the angiotensin II receptor type 1-specific blocker, olmesartan treatment, could be a potential therapy in treating ER stress-induced renal apoptosis.<sup>23</sup>

#### Chemical Chaperones

Chemical chaperones are small molecules that

stabilize the folding of proteins and buffer abnormal protein aggregation.<sup>24</sup> These compounds include 4-phenylbutyrate and tauroursodeoxycholic acid.

#### Tauroursodeoxycholic Acid

Tauroursodeoxycholic acid is an amphiphilic bile acid. It is the taurine conjugate form of ursodeoxycholic acid, which is produced in several countries for the treatment of gallstones and liver cirrhosis. Having beneficial effects in cardiac dysfunction, Huntington disease, Parkinson disease, and stroke, TUDCA has diminishing apoptotic effects.<sup>25</sup> Tauroursodeoxycholic acid prevents apoptosis in the Bax pathway. It prevents the Bax molecule from being transported to the mitochondria to release cytochrome C. This protects the mitochondria from perturbation and the activation of caspases.<sup>26</sup> Tauroursodeoxycholic acid acts as a chemical chaperone.

#### 4-Phenylbutyrate

4-Phenylbutyrate can suppress oxidative stress by attenuating ER stress. Luo and colleagues showed that administration of 4-phenylbutyrate inhibited expression of inositol-requiring enzyme-1 $\alpha$  and nuclear factor (erythroid-derived 2)-like 2-related factor 2 in rats' renal tissue with streptozotocin-induced DN.<sup>27</sup>

#### Renal Epidermal Growth Factor Receptors

Renal epidermal growth factor receptors are activated in models of DN. Zhang and colleagues examined the effect of treatment with erlotinib, an inhibitor of epidermal growth factor receptor activity, on the progression of DN in a type 1 diabetic mouse model. Erlotinib-treated animals had less histological glomerular injury and had decreased ER stress. This study demonstrated that inhibition of epidermal growth factor receptor with erlotinib attenuated the development of DN in type 1 diabetes mellitus, which is mediated at least in part by inhibition of ER stress.<sup>28</sup>

#### ENDOPLASMIC RETICULUM STRESS AND RENAL TUBULAR CELLS

Endoplasmic reticulum stress has also been demonstrated in the tubulointerstitial compartment of patients with progressive DN.<sup>29</sup> Several studies showed the impact of ER stress on renal tubular epithelial cells in DN. Liu and coworkers showed that

upregulation expression of *GPR78* was involved in premature senescence of renal tubular epithelial cells in DN.<sup>30</sup> Watanabe and colleagues showed similar results.<sup>31</sup> On the other hand, chemical chaperone TUDCA is an enhancer for the adaptive capacity of ER stress induced by albuminuria in DN.<sup>32</sup>

Apoptosis of tubular epithelial cells accompanies DN. Brezniceanu and colleagues observed greater expression of caspase-12 in the proximal tubules of the diabetic mice compared with the nondiabetic mice. Albumin stimulates activity of both caspase-12 and caspase-3 as well as the CHOP protein in a human proximal tubule cell line (human kidney 2). Furthermore, knockdown of caspase-12 with small interfering RNA reduced albumin-induced apoptosis in human kidney 2 cells.<sup>33</sup>

#### THERAPEUTIC ASPECTS

Several studies have regarded therapeutic aspect of ER stress in DN. It was shown that aliskiren, a direct renin inhibitor, and valsartan, an angiotensin II type 1 receptor blocker, provided protection in a model of DN in mice. At once, they significantly prevented increased expression of ER stress markers (CHOP and XBP1) in the kidney of diabetic animals. They concluded that aliskiren and valsartan protected against diabetic kidney disease through multiple mechanisms, including ER stress.<sup>34</sup>

Lim and coworkers showed that the endocannabinoid system is involved in the onset of DN. Hyperglycemia significantly increased cannabinoid receptor 1, mRNA, and protein levels. Hyperglycemia also induced increases in ER stress proteins and apoptosis. These apoptotic effects were prevented by cannabinoid receptor 1 antagonist (AM251). Therefore, they suggest that blockade of cannabinoid receptor 1 may be a potential therapy in DN.<sup>35</sup>

#### CONCLUSIONS

Diabetic nephropathy is the major leading cause of end-stage renal disease. Pathogenesis of DN has been widely studying. Many of them show the role of ER stress in it. Free fatty acids, advanced glycated end products, autophagy, and angiotensin II receptor pathway are known to be involved in ER stress-mediated pathogenesis of DN. Some of them display how chemical chaperones can modify ER stress.

**CONFLICT OF INTEREST**

None declared.

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