Stress in the kidney is the road to pERdition: is endoplasmic reticulum stress a pathogenic mediator of diabetic nephropathy?

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Abstract

The endoplasmic reticulum (ER) is an organelle that primarily functions to synthesise new proteins and degrade old proteins. Owing to the continual and variable nature of protein turnover, protein synthesis is inherently an error-prone process and is therefore tightly regulated. Fortunately, if this balance between synthesis and degradation is perturbed, an intrinsic response, the unfolded protein response (UPR) is activated to restore ER homoeostasis through the action of inositol-requiring protein 1, activating transcription factor 6 and PKR-like ER kinase transmembrane sensors. However, if the UPR is oversaturated and misfolded proteins accumulate, the ER can shift into a cytotoxic response, a physiological phenomenon known as ER stress. The mechanistic pathways of the UPR have been extensively explored; however, the role of this process in such a synthetic organ as the kidney requires further clarification. This review will focus on these aspects and will discuss the role of ER stress in specific resident kidney cells and how this may be integral in the pathogenesis and progression of diabetic nephropathy (DN). Given that diabetes is a perturbed state of protein turnover in most tissues, it is important to understand if ER stress is a secondary or tertiary response to other changes within the diabetic milieu or if it is an independent accelerator of kidney disease. Modulators of ER stress could provide a valuable tool for the treatment of DN and are under active investigation in other contexts.

Key Words
- endoplasmic reticulum stress
- diabetic nephropathy
- mTORC1
- podocyte
- proximal tubule cell

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Introduction

A tightly balanced homoeostasis exists between the synthesis and degradation of proteins, which is maintained in the endoplasmic reticulum (ER). When imbalance occurs in either one or both of these facets, the ER activates coordinated adaptive responses known as the unfolded protein response (UPR) and the ER-associated degradation (ERAD) pathway in an attempt to restore balance in the ER. However, when these responses are saturated, the misfolded proteins accumulate in the ER, and cells develop a detrimental condition referred to as ER stress. The principles of the UPR are now relatively well defined (Ron & Walter 2007, Hasnain *et al.* 2012, Hetz 2012), and the mechanisms of the signal transducers involved in the activation of this pathway have already been the subject of detailed reviews (Ron & Walter 2007). However, several key questions remain

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unanswered: i) how the UPR system integrates within specific resident cells of the kidney, ii) how diseases such as diabetes can promote an imbalance at these sites and iii) whether this ultimately leads to ER stress-induced pathology culminating in nephropathy. This review summarises recent developments in these areas and highlights new insights into translational applications into the clinical environment.

Function of the ER

The ER functions as a site of synthesis, folding and maturation of secreted, luminal and transmembrane proteins. Synthesis of unfolded polypeptide chains occurs in the rough ER where they undergo a maturation process, mediated by ER-resident enzymes and chaperones to form a stoichiometric stable conformation that is both energetically efficient and biologically active (Dobson 2003, Araki & Nagata 2011).

During the synthesis of new proteins, the stoichiometry of the amino acid chains can be altered by post-translational modifications, thereby profoundly affecting the energy signature and conformation of the folded protein. The role of post-translational modifications in the development of disease is becoming more apparent, particularly in chronic conditions such as diabetes (van Lummel *et al.* 2013), due to their effects on the generation of inappropriately folded proteins, observed most vividly in congenital disorders of glycosylation (Cylwik *et al.* 2013). Subsequently, the maturation of these nascent proteins is further dependent on the appropriate intracellular concentrations of calcium, glucose and ATP, as well as the redox environment (Kaufman *et al.* 2002); all of which function as ER quality-control factors. When proteins are both correctly folded and carry the appropriate post-translational modifications, the protein exits the ER and progresses through the secretory pathway to the Golgi body. When folded inappropriately, proteins accumulate within the ER, where UPR pathways are activated or terminally misfolded proteins are retro-translocated from the ER into the cytoplasm and undergo proteasome degradation in the ERAD pathway.

Protein homoeostasis within cells is particularly complex in nature as a balance is forged among constant turnover, the synthesis of new proteins and the degradation of old proteins, as well as the influx of new proteins and precursors from the extracellular environment. Unfortunately, protein synthesis is inherently an error-prone process. Nevertheless, the dynamic nature of the ER allows cells to adjust their protein-folding capacity in response to fluctuations in the environment through transmembrane sensors that face the ER lumen and effectors that signal to other compartments of the cell, ensuring that secreted proteins are maintained at a high fidelity and at the correct concentrations to maintain protein homoeostasis.

Triggering the UPR

In mammalian cells, there are three major UPR pathways, activated by transmembrane sensors located in the ER membrane. These three major pathways are the inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6) and the PKR-like ER kinase (PERK)-mediated response. When protein synthesis and degradation in the ER are under homoeostatic balance, these three sensors are inactive and are suggested to be either inherently inactive until unfolded proteins activate the sensors, maintained in an inactive state by the ER chaperone BiP, or regulated by hybrid of both of these mechanisms (Ron & Walter 2007). When activated, these pathways elicit several physiological responses in order to counteract the increased accumulation of inappropriately folded proteins. Ultimately, the purpose of the UPR pathway is to prevent an overabundance of misfolded proteins in the ER, which would consequently result in ER stress and potentially cell death.

As misfolded proteins begin to accumulate in the ER, pathways of the UPR are activated. The first identified stress transducer IRE1 (Cox *et al.* 1993, Mori *et al.* 1993) undergoes oligomerisation following the accumulation of misfolded proteins and *trans*-autophosphorylates, which allosterically activates IRE1 endoribonuclease activity via a conformational change (Papa *et al.* 2003). IRE1-mediated sequence-specific cleavage is targeted at X-box-binding protein 1 (*XBP1*) mRNA causing a frame shift in the coding sequence. A frame shift in *XBP1* mRNA leads to the manufacture of the potent transcriptional activator (*XBP1s*), which activates genes encoding ER chaperones, enzymes that promote protein folding, maturation, secretion and ERAD components in an attempt to decrease ER load and relieve ER stress (Hollien & Weissman 2006, Glimcher 2010). In parallel, ATF6 traffics to the Golgi in the presence of misfolded proteins, where it is cleaved by proteases S1P and S2P (Ye *et al.* 2000) revealing a cytosolic fragment (ATF6*n*), which has a DNA-binding domain. ATF6*n* subsequently migrates to the nucleus and in a similar manner to *XBP1s*, functions as a transcriptional

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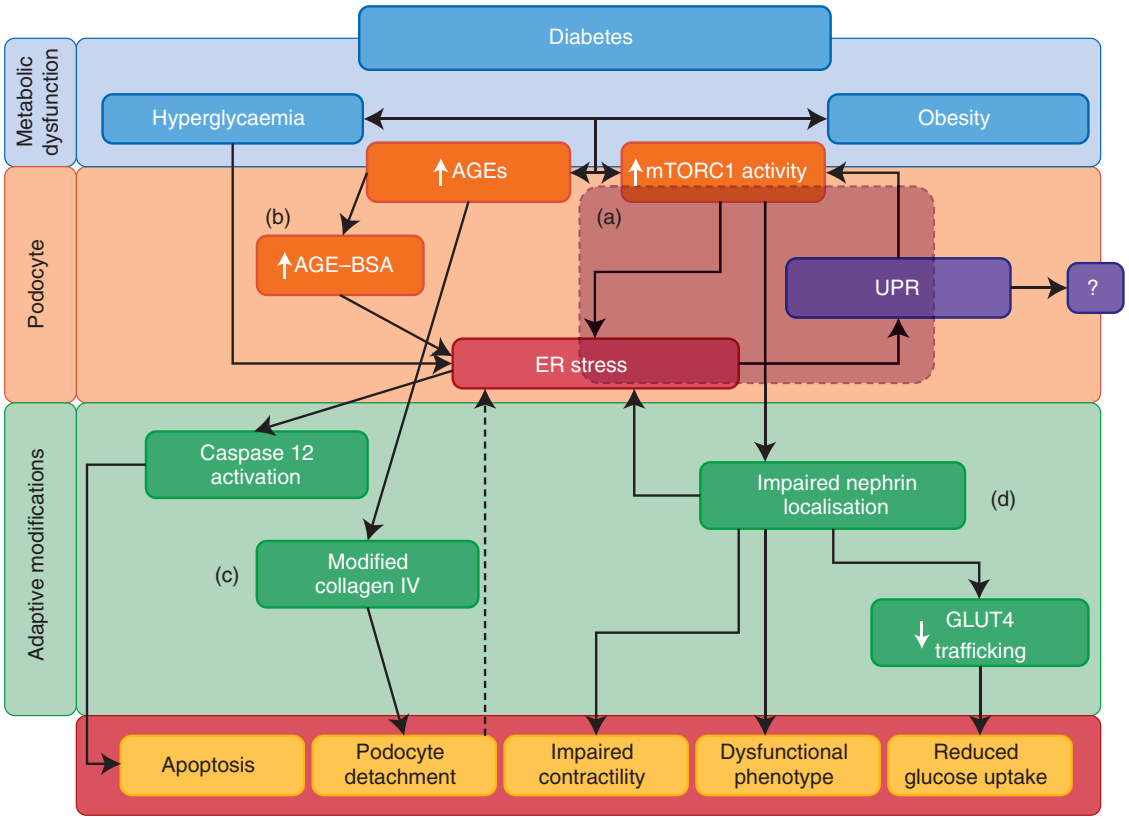

Figure 1

A summary of the proposed mechanisms of ER stress-induced podocyte dysfunction. (a) Glomerular stress events, mediated by diabetes in this case, and the subsequent metabolic and catabolic changes perturb the balance among mTORC1, the ER and UPR. (b) AGE–BSA induces podocyte apoptosis mediated by caspase 12 activation (Chen *et al*. 2008, 2011). (c) AGE-modified collagen IV contributes to podocyte detachment or dysfunction (Chuang *et al*. 2007) and ER stress leading to the development of podocyte dysfunction. Obesity is probably a factor contributing to dysfunctional mTORC1 activity, given that mTORC1 activity is increased in the glomerulus in obese *db/db* mice with a targeted genetic insertion of mTORC1 activity when compared with *db/m* mice (Inoki *et al*. 2011). (d) Chronic changes in mTORC1 activity could be a determinant pathway leading to the development of diabetic nephropathy through various pathological changes to the podocyte (Coward *et al*. 2007, Inoki *et al*. 2011).

activator for proteins that assist in resolution of ER stress by increasing ER capacity (Haze *et al*. 1999).

Unlike the other two signal transducers, PERK phosphorylation activates a substrate known as eukaryotic translation initiation factor 2α (eIF2α). Activation of eIF2α reduces ER stress not by increasing the ER potential, rather eIF2α reduces the load on the ER by inhibiting translation of new proteins from mRNA, leading to a reduction in overall protein synthesis through the inhibition of AUG codon recognition at the ribosomal level (Harding *et al*. 1999).

The pathways of the UPR are essential in maintaining normal physiological functions, particularly in cells with a high rate of protein synthesis (kidney tubule cells (Goldspink & Kelly 1984, Tessari *et al*. 1996)) or secretory function (pancreatic β-cells and liver cells (Rutkowski & Hegde 2010)). Additionally, as the UPR is conserved through many species, perhaps the design of this pathway was intended to be acutely beneficial dependent on the disease conditions, ameliorating damage. Unfortunately, when the UPR is consistently activated such as in the presence of a chronic condition (such as diabetes) or during viral infection where cellular protein machinery is a means for viral replication, this adaptive response of the UPR system develops into a cytotoxic response promoting increased autophagy and apoptosis in an attempt to halt disease progression.

ER stress in diabetes

The ER stress response is thought to be involved in several pathophysiological developments throughout the progression of diabetes and the associated chronic complications. The relationship between ER stress

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and the regulatory metabolic pathways in the ER is incredibly diverse. However, as the current literature stands, there appears to be a greater focus on ER stress and the downstream response in type 2 diabetes (T2D) when compared with T1D, although this does not reflect the lack of relevance to this disorder as well as its role, in the development of complications associated with diabetes.

Type 2 diabetes

Obesity is a common comorbidity in individuals with T2D characterised by dysregulation of hepatic glucose production in combination with declining peripheral insulin sensitivity in the face of inadequate insulin secretion by the pancreatic β-cells. During the progression of T2D, there is an increased demand on the β-cells for insulin production in order to compensate for the ongoing intolerance. Moreover, an unfortunate eventuality for the majority of obese T2D is the advent of both glucotoxicity and lipotoxicity, where the imbalance between glucose and lipid homoeostasis is a confounding result of nutrient excess as well as other comorbidities such as renal or hepatic disease.

Initial studies implicating ER stress in the pathogenesis of T2D indicated that genetically obese mice (*ob/ob*) or mice fed on a high-fat diet inducing obesity had increased parameters of the UPR in the liver and adipose tissues (Ozcan *et al.* 2004). Similarly in insulin-resistant, non-diabetic humans, there are marked increases in the presence of the IRE1 (ERN1) protein, an indicator of the adaptive response of the UPR (Boden *et al.* 2008). Despite only detecting changes in one arm of the UPR in the aforementioned study, another study cohort of severely obese patients who underwent surgery-assisted weight loss (BMI of 51.3 compared with a BMI of 33.5) exhibited significant decreases in all aspects of the UPR arms in the majority of patients, indicating a significant reduction in ER stress after gastric-bypass-induced weight loss (Gregor *et al.* 2009). Noteworthy were the concomitant improvements in overall metabolic health afforded to these patients with the associated surgery. Evidently, certain polymorphisms in the *ATF6* gene were identified to be associated with T2D and modulation of plasma glucose in certain populations of the Dutch (Meex *et al.* 2007) and Pima Indians (Thameem *et al.* 2006). Unfortunately, among indigenous populations, specifically the Pima Indians (Nelson *et al.* 1996, Lemley 2003) and Australian Aborigines (Hoy *et al.* 1999, McDonald & Russ 2003), there is an inherent increased prevalence of diabetic comorbidities of both cardiovascular and renal diseases. Whether genetic polymorphisms resulting in dysfunctional responses to ER stress could provide a basis accompanied by other factors for the accelerated progression of renal disease needs to be further established in these populations. These genetic associations are not constitutive among all ethnic backgrounds, with very little association noted among the certain population groups of the Chinese (Chu *et al.* 2007, Hu *et al.* 2011), Caucasian (Chu *et al.* 2007) and African-American (Chu *et al.* 2007) ethnicities.

Prolonged exposure to nutrient excess in obese individuals promoting an environment of systemic ER stress could synergistically contribute to the pathophysiology of both insulin resistance and hyperglycaemia in obesity and T2D. Each of these would clearly affect diabetic kidney disease where there are clear benefits of strict glycaemic (Patel *et al.* 2008, Gerstein *et al.* 2008) and lipid control (Fried *et al.* 2001, Collins *et al.* 2003), as well as weight loss and caloric restriction (Gross *et al.* 2002, Ros *et al.* 2004). However, it is not certain whether ER stress is a consequence of diabetes or the developed comorbidity of obesity.

Type 1 diabetes

The traditional notion of T1D as primarily an autoimmune disease in humans is currently being challenged and it is being tentatively labelled as an inflammatory pancreatic-wide disease (Skog *et al.* 2013). Therefore, this hypothesis raises interesting possibilities that other damage mechanisms such as ER stress as act pathogenic mediators of T1D, given that islets isolated from individuals with T1D have increased levels of ER stress markers (Marhfour *et al.* 2012). A particularly interesting notion is the integral role of PERK, an ER stress signalling molecule, in maintaining normal pancreatic health and function. Murine models with null mutations in *Perk* (*Eif2ak3*) and mutations at the Ser51 phosphorylation site in *Eif2α* (*Eif2s1*) exhibit β-cell dysfunction and diabetes (Harding *et al.* 2001, Scheuner *et al.* 2001, 2005). Additional work has described the necessity for PERK function during the neonatal stage in pancreas development (Zhang *et al.* 2006). Not only does PERK appear to be essential during the development of the pancreas, but there is evidence that maintenance of normal β-cell function in the adult pancreas also requires PERK (Gao *et al.* 2012). Furthermore, a mutation in the *EIF2AK3* gene causes an autosomal recessive disease known as Wolcott–Rallison syndrome in humans, who often exhibit presentation of T1D and renal insufficiencies during the neonatal period

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(Iyer *et al*. 2004). Interestingly, within the South Indian population, polymorphisms between the regions around the *EIF2AK3* locus show an association with T1D susceptibility (Allotey *et al*. 2004). Evidently when compared with the neighbouring regions, the South Indian population has the highest prevalence of kidney disease (Rajapurkar *et al*. 2012). It has also been observed that South Indian/Asian migrants have a greater prevalence and faster progression of diabetic nephropathy (DN) when compared with their European counterparts (Samanta *et al*. 1986, Chandie Shaw *et al*. 2006). This is particularly noteworthy as it may indicate that in the advent of ER stress a deficiency in PERK function could play a contributing role in the progression of comorbidities, such as DN.

ER stress and DN

The role of ER stress in diabetic injury is not a new concept, with several indications that the presence of high glucose and free fatty acids in diabetic patients can induce ER stress, implicating ER stress in the development of complications such as retinopathy (Oshitari *et al*. 2008, Jing *et al*. 2012) and cardiovascular disease (Beriault & Werstuck 2012, Xu *et al*. 2012). However, it has only been recently demonstrated that there has been a focus on the potential role of ER stress in the pathogenesis of DN, with reports identifying the activity of the UPR in podocytes and other kidney cells (Cheng *et al*. 2006, Liu *et al*. 2008, Tao *et al*. 2012). Lindenmeyer *et al*. (2008) identified the induction of *UPR* genes in humans with DN in a manner that is dependent on the degree of kidney injury, where biopsies of patients with established DN exhibited increased levels of XBP1 and ER chaperones (HSPA5/GRP78 and HYOU1/ORP150), when compared with those with only mild diabetes. Moreover, much like the pancreatic β-cells, the kidneys could constitutively be a site sensitised for the induction of ER stress, due to the high rates of protein synthesis. Although surprising, in humans, it is estimated that the fractional rates of protein synthesis by the kidneys is approximately 42% of the total body load daily (Tessari *et al*. 1996), which is the highest in the body, further indicating that kidney cells could be highly susceptible to ER stress, due to fluctuations in the ER load. It is important to identify the disparate functions of the different resident kidney cells, and understanding how ER stress contributes to DN pathophysiology in the unique cell populations may elucidate novel pharmacological targets for remediating the disease.

ER stress-mediated glomerular injury

Glomerular epithelial cells (podocytes) play an essential role in the health and function of the glomeruli. The podocytes are insulin-sensitive cells (Coward *et al*. 2005, Welsh *et al*. 2010) that contract to limit diuresis, fluxing nutrients into the urine for the primary purpose of nutrient retention and post-prandial utilisation. Unfortunately, the podocyte is a terminally differentiated cell with limited capabilities to regenerate and proliferate following injury, therefore reduced podocyte density is argued to be a crucial determinant in the development and progression of DN (Pagtalunan *et al*. 1997, Susztak *et al*. 2006). The induction of ER stress has been elegantly reviewed in primary glomerular diseases (Dickhout & Krepinsky 2009). However, developing a greater understanding as to how ER homoeostasis is perturbed in these cells during diabetes and the subsequent adaptive responses is warranted.

Mechanisms culminating in a stressed diabetic podocyte ER

Currently, there are no direct studies investigating the specific role that ER stress plays through the targeted deletion of the ATF6/IRE1/PERK pathways in the podocytes, irrespective of diabetic conditions. Common insults such as the accumulation of advanced glycation end products (AGEs) and the activation of their receptors have been implicated in not only the pathogenesis of DN (Forbes *et al*. 2003, Wendt *et al*. 2003, Penfold *et al*. 2010, Tan *et al*. 2010) but also the induction of ER stress (Inagi *et al*. 2005, Cheng *et al*. 2006). Moreover, the accumulation of AGEs within the skin (representing tissue accumulation) also predicts those T1D individuals at a greatest risk of developing nephropathy (Yu *et al*. 2006). Additionally albumin, a major component in the proteinuria observed in DN, is often reflective of progressive disease associated with a declining glomerular filtration barrier and increased leaching into the urine. Not only could excessive exposure of podocytes to albumin induce ER stress pathways leading to caspase-12-mediated podocyte apoptosis independent of mitochondrial input (Chen *et al*. 2011), but also it appears that the modification of albumin by AGEs enhances pro-apoptotic pathways in podocytes in a dose- and time-dependent manner (Chen *et al*. 2008). Moreover, Chuang *et al*. (2007) observed that both soluble and matrix-bound AGEs induced apoptosis in podocytes in an additive manner and they further observed an increase in podocyte detachment when they were cultured in the presence of AGE-modified

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collagen IV. This is of particular interest as this may implicate not only the number of podocytes as a major determinant in the development and progression of DN but also their integrity, which has been well described (Sachs & Sonnenberg 2013). Moreover, parallels can be drawn from this postulation, with the identification that the ability for the podocyte to effectively adhere to the extracellular matrix is a major determinant of ER stress induction and the subsequent development of glomerular injury (Cybulsky *et al.* 2011). This may indicate that with the onset of podocyte dysfunction, ER stress could potentially exacerbate damage to the cell.

Moreover, it is suggested that during the advent of hyperglycaemia in diabetic patients, the balance of the ER load in the podocyte is perturbed through a number of systemic pathways leading to the induction of ER stress and subsequently podocyte injury. Indeed, ER stress is induced in podocytes when acutely stimulated under both high-glucose (Cao *et al.* 2014) and low-glucose (Fujii *et al.* 2006) conditions, and it is apparent that there are regulatory mechanisms to alleviate this stress when podocytes are chronically exposed to hyperglycaemic conditions (Inagi *et al.* 2005). Given that cell survival is promoted through the activation of autophagy in the advent of ER stress induced in mammalian cells in a high-glucose environment (Ogata *et al.* 2006), it is likely that compensatory modifications to the ER could be directly linked to autophagy.

Adaptive responses to a perturbed ER in the diabetic podocyte

Autophagy, a highly regulated lysosomal pathway, selectively targets and removes damaged organelles and is suspected to further function in tandem with the ER as a compensatory mechanism for proteasomal degradation (Ding *et al.* 2007). Not only is autophagy implicated to function in the adaptation of stress and survival mechanisms, but also autophagy may play a distinctive role in the metabolic regulation of a cell. It appears that autophagy intricately regulates lipid metabolism (Singh *et al.* 2009*a*,*b*) and is involved in the degradation of large glycogen stores in the hepatocytes of neonates (Kalamidas *et al.* 2004). Interestingly, podocytes maintain a constitutively higher level of autophagy when compared with other intrinsic renal cells (Asanuma *et al.* 2003, Hartleben *et al.* 2010). This may simply be due to the limited capacity for regeneration of the podocyte and hence requirement to constantly recycle and renew cellular components. However, there may be an underlying involvement of autophagy in the metabolic capacity of the podocyte.

Recent developments in the understanding of the function of the protein kinase mammalian target of rapamycin complex 1 (mTORC1) in podocytes have identified a relationship between autophagy and ER function. mTORC1 is a kinase complex involved in a myriad of cellular processes including a modulatory role of autophagy in response to nutrients such as glucose, amino acids and growth factors (Hay & Sonenberg 2004). Interestingly, there is evidence to suggest that much like mTORC1, the UPR can be activated through various physiological stimuli (circulating FFAs (Pineau & Ferreira 2010), hypoxia (Wouters & Koritzinsky 2008) and growth stimuli (Christis *et al.* 2010, Pfaffenbach *et al.* 2010)) that do not necessarily act through the accumulation of unfolded proteins in the ER. Moreover, a recent review (Appenzeller-Herzog & Hall 2012) has elaborately summarised how mTORC1 operates both upstream and downstream of ER stress signals, essentially inferring an intricate relationship among ER stress, autophagy and mTORC1. The speculation that mTORC1 may be involved in the pathogenesis of DN came from the observation that an inhibitor of mTORC1 (rapamycin) attenuates renal hypertrophy in experimental models of DN (Sakaguchi *et al.* 2006). Rapamycin selectively restores integrity of the podocyte foot processes through the stabilisation of the protein nephrin in the filtration barrier and reduces subsequent tubular and glomerular damage (Inoki *et al.* 2011, Kato *et al.* 2012). Nephrin is an integral podocyte protein involved in the retention of larger macromolecules in the blood as well as playing a role in the membrane docking of the insulin-sensitive glucose transporter, GLUT4 (Coward *et al.* 2007). However, the changes to the role and function of mTORC1 in podocytes, in response to the metabolic alterations that occur during diabetes, remain to be completely delineated.

It has been suggested, however, that mTORC1 activation in podocytes may play an essential role in the development of DN, as podocyte-specific mTORC1 activation in the absence of diabetes recapitulated several features of DN (Inoki *et al.* 2011). Coupled with the chronic activation of mTORC1 in the podocytes, ER stress was enhanced in the glomeruli and the pathological changes in the podocyte could partially be attributed to ER stress (Inoki *et al.* 2011). When mTORC1 activity was genetically reduced in diabetic mice, there was a significant reduction in the development of DN (Inoki *et al.* 2011). Consistent with the ideology that the activation of the UPR can occur both downstream and upstream of mTORC1 infers a particularly interesting notion that, in certain contexts, mTORC1 is a component of the process of ER stress-induced cell toxicity, and if perturbed in the

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podocyte, could be crucial to the development of DN. Indeed, despite the fact that hyper-activation of mTORC1 in mouse podocytes led to a phenotype akin to that of DN, reducing mTORC1 activity similarly promoted podocyte dysfunction (Godel *et al*. 2011), indicating that mTORC1 activity is essential for the development of the podocyte. Interestingly, Inoki *et al*. (2011) determined that mTORC1 was causing podocyte dysfunction by retention of nephrin in the cytoplasm, where it could not localise to the renal filtration barrier during hyper-activation of mTORC1 in podocytes. Not only would impairments and the subsequent accumulation of dysfunctional nephrin promote ER stress (Drozdova *et al*. 2013), but also a decline in nephrin is known to lead to the development of proteinuria (Langham *et al*. 2002) and glucose homoeostatic defects in podocytes. As mentioned above, a loss of nephrin also impairs GLUT4 trafficking to the plasma membrane and therefore insulin-dependent glucose uptake (Coward *et al*. 2007). However, the mechanism by which mTORC1 activity alters the capacity of nephrin to localise to the glomerular filtration barrier needs to be further explored. One possible avenue would be to identify the effects of post-translational modifications of nephrin in the ER and Golgi, which could alter nephrin localisation and function. One particular modification of interest is *N*-glycosylation, which plays a critical role in mediating membrane localisation of nephrin (Yan *et al*. 2002) and occurs within the ER. Hence, impaired protein modification in the ER, either through mechanisms directly or indirectly associated with ER stress, could also promote podocyte dysfunction and hence influence the progression of DN.

These data illustrate a rather intricate relationship between ER stress and mTORC1 activity (Fig. 1). Although there is growing body of evidence indicating the crucial role for the mTORC1 pathway in podocyte health, unfortunately, at present, there is a limited understanding of the intersecting relationship among ER stress, mTOR and the UPR in the development of human DN.

ER stress-mediated tubulointerstitial injury

Tubulointerstitial damage is considered a final common pathway to end-stage kidney disease, where the deposition of extracellular matrix, oedema and infiltrating cells separate the proximal tubules from their intimate contact with the renal tubular capillaries. As one of the primary functions of the proximal tubule is the reabsorption of substances, such as glucose and sodium, from the urinary filtrate and their return into the bloodstream, tubulointerstitial fibrosis is the best prognostic indicator of progression to end-stage renal disease requiring transplantation or dialysis. However, in patients with diabetes, renal biopsies are no longer performed routinely due to an increased risk of bleeding and poor wound healing within this population. Proximal tubular epithelial cells (PTCs) are another particular type of cells of interest in the pathophysiology of kidney tubular injury in DN (Remuzzi & Bertani 1998), particularly as they are highly susceptible to ER stressors (Zinszner *et al*. 1998) and are highly synthetic and metabolically active cells.

Mechanisms culminating a stressed diabetic PTC ER

A common hallmark of DN is proteinuria, specifically the loss of albumin (albuminuria), and one of the central roles of the PTCs is the ability to reabsorb a proportion of proteins that may be filtered by the glomerulus and consequently end up within the urinary filtrate, which is exacerbated in the context of diabetes. Moreover, several studies show a direct association between proteinuria and pathogenesis of tubular injury, with the levels of albumin and modified albumin as the determinant factor (Ruggenenti *et al*. 1998, Coughlan *et al*. 2011). The effects of albumin on PTCs are biphasic, where on one hand albumin stimulates the growth of PTCs (Iglesias *et al*. 1999, Dixon & Brunskill 2000) while on the other hand, excessive exposure to albumin is implicated in tubular atrophy in a pro-apoptotic and pro-inflammatory environment (Ohse *et al*. 2006). Moreover, albumin-induced ER stress is implicated in causing tubular damage through the activation of caspase 12 subsequently resulting in apoptosis of PTCs (Ohse *et al*. 2006). Excessive reabsorption of albumin has also been suggested to promote reactive oxygen species production and activation of PPARγ, stimulating GRP78 and eIF2α phosphorylation, both of which are markers of ER stress. ER stress subsequently stimulated downstream phosphorylation of JNK and NFκB, resulting in increased expression of sodium-dependent glucose transporter 2 (SGLT2 (SLC5A2)) in cultured rabbit PTCs (Lee *et al*. 2009). Increased SGLT2 expression has previously been identified in PTCs isolated from the urine in T2D patients (Rahmoune *et al*. 2005). However, mice that have the expression of *Sglt2* ablated in the PTCs are not protected against the development or progression of kidney injury (Vallon *et al*. 2013). Whether increased expression of SGLT2 correlates with an increased risk of the development of DN is yet to be determined. Nevertheless, given the important role of SGLT2 in glucose reabsorption in the

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proximal tubule and the advent of new therapies in diabetic treatment regimens that target this protein to control hyperglycaemia by inducing glycosuria (Bailey *et al*. 2010, Wilding *et al*. 2012), these pathways involving ER stress warrant further investigation and understanding.

Adaptive responses to a perturbed ER in diabetic PTCs

What is particularly interesting to note is the identification of mTORC1 as an upstream mediator in PTCs, before the induction of ER stress as a result of excess albumin exposure (Lee *et al*. 2011), although whether albumin derived from human sera functions in a similar manner is yet to be determined. Similar to podocytes, mTORC1 may also play a crucial role in the pathophysiology of kidney tubular injury through the regulation of ER stress. Interestingly, it has been further elucidated, in tubular cells, that activation of pro-apoptotic pathways can be mediated by the induction of ER stress. Not only was mTORC1 determined to function downstream of ER stress in these cells, but subsequent mTORC1 activity involved pathways culminating in IRE1–JNK activation (Kato *et al*. 2012). Interestingly, inhibition of mTORC1 with rapamycin under the same physiological conditions selectively inhibited the IRE1 pathway. This further implies mTORC1 activity both upstream and downstream of ER stress; however, the reason that mTORC1 selectively activates the IRE1 branch and not the other branches of the UPR needs to be further elucidated. One interpretation (Fig. 2) suggests that albumin may direct ER stress in the proximal tubules of diabetic patients with albuminuria, mediating reduced insulin responsiveness in the PTCs through IRE1–JNK activation driven by mTORC1, in which the IRE1–JNK pathway has been shown to reduce insulin sensitivity in adipocytes, although mTOR activity was decreased in the adipose tissue of the T2D patients (Li *et al*. 2014). Reduced mTOR activity could simply be a consequence of developed insulin resistance, as sustained activation of mTORC1 renders insulin receptor signalling pathways irresponsive to insulin (Shah *et al*. 2004). Ultimately whether mTORC1 activity would reduce insulin sensitivity in PTCs needs to be confirmed. However, the question of how insulin is relevant to the PTCs is an evolving concept, given that these cells are not physiologically insulin-sensitive cells with respect to glucose uptake, nor do they manufacture the enzymes necessary to perform glycolysis under normal conditions (Vallon 2011). Recent research has revealed that indeed PTCs require insulin receptor-mediated signalling for their normal function, and when the insulin receptor was specifically deleted in the PTCs, mice exhibited increased renal gluconeogenesis independent of glucose clearance, as well as enhanced insulin secretion and action (Tiwari *et al*. 2013). It is postulated that the downregulation of insulin receptor and/or insulin resistance in the PTCs could further contribute to hyperglycaemia, which could effectively enhance the risk for the development of DN. Although the mechanisms and actions of mTORC1 remain to be further explored in these cells, mTORC1 activity may play a central role not only in tubular injury via changes in insulin receptor signalling but also in the development of DN.

Therapeutic implications of ER stress modulators

Current approaches to treat DN largely target systemic blood pressure and/or intraglomerular hypertension. More often than not the first line of therapies for the treatment of DN are those that influence the renin–angiotensin system, including angiotensin-converting enzyme inhibitors (Lewis *et al*. 1993) and angiotensin II receptor antagonists (Brenner *et al*. 2001), with concurrent glycaemic control agents and often therapies for hyperlipidaemia. Despite these interventions, progression of DN to end-stage renal disease can only be slowed but not cured. Although these interventions are currently the most effective techniques for clinical management of microvascular complications such as nephropathy, there is also evidence to suggest that strict glycaemic control does not necessarily reduce the risk of cardiovascular disease (Gerstein *et al*. 2008) and may potentially elevate the risk of a cardiovascular event (Patel *et al*. 2008). Moreover, an unfortunate phenomenon often observed in the treatment of diabetes and its complications is poor adherence to treatment regimens, particularly with the rates of adherence inversely proportional to the number of diabetic medications prescribed (Cramer 2004). This provides a rationale for the necessity of developing not only novel therapeutics that can target multiple pathways, but also therapeutics that assist in improving patient compliance as well as addressing other pathogenic mediators. A potential avenue to assist in achieving this therapeutic goal may be to target ER stress pathways.

There are a myriad of compounds (Table 1) that improve ER folding capacity such as the chemical chaperones 4-phenylbutyric acid (PBA), taurine-conjugated ursodeoxycholic acid (TUDCA) and ER chaperones that reduce ER stress and improve insulin action and sensitivity (Ozawa *et al*. 2005, Ozcan *et al*. 2006). Small-molecule

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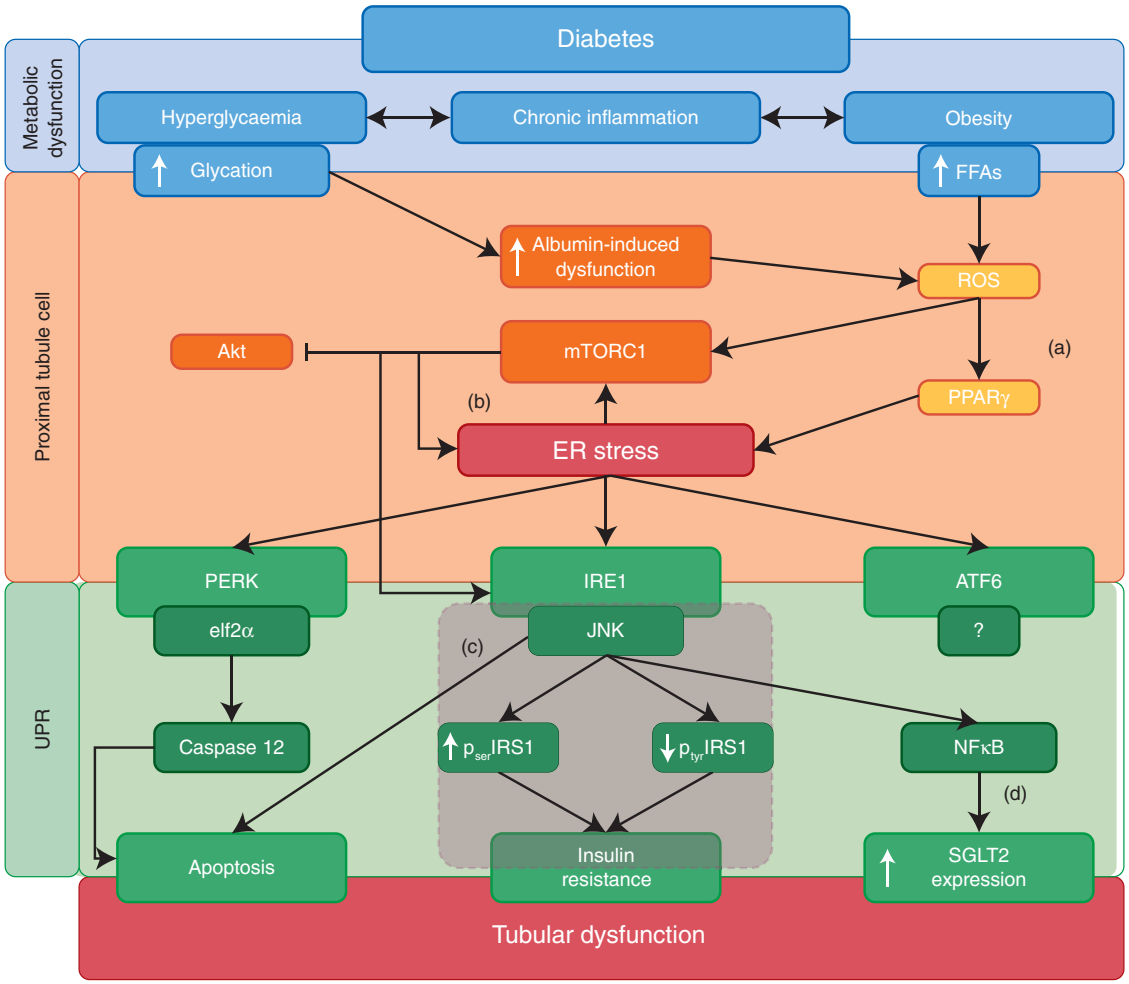

Figure 2

A summary of the proposed mechanisms of ER stress-induced tubular dysfunction. Metabolic imbalances in diabetes (hyperglycaemia, inflammation and obesity) contribute to the interacting pathways of ER stress contributing to tubular dysfunction. Enhanced glycation is directly associated with pathways implicated in the development of diabetic nephropathy (DN) through the modification of albumin generating large macromolecular complexes (Penfold *et al*. 2010). (a) Excessive reabsorption of albumin is thought to overload the PTCs leading to ER stress-induced apoptosis mediated by caspase 12 activation (Ohse *et al*. 2006). (b) Moreover, albumin appears to have an intricate signalling pathway both upstream and downstream of ER stress mediated by mTORC1. Enhanced mTORC1 activity in the PTCs not only contributes to pro-apoptotic pathways, but through the selective activation of the IRE1–JNK pathway could also lead to decreased responsiveness to insulin in the PTCs. (c) However, it is important to note that the proposed pathway leading to IRE1–JNK-induced insulin resistance was only identified in adipocytes from T2D patients (Li *et al*. 2014) and has not been substantiated in PTCs. (d) Furthermore, JNK activation could further promote metabolic imbalances in the PTCs through ER stress-induced NFκB stimulation of SGLT2 expression (Lee *et al*. 2009). ER stress might perturb the metabolic integrity of the proximal tubule, which could be a crucial pathway contributing to the pathogenesis of DN.

chemical chaperones such as TUDCA and 4-PBA are suspected to enhance either protein secretion or the folding capacity of the ER (Park & Ozcan 2013). Indeed, recent work on experimental models of DN has demonstrated that 4-PBA (Qi *et al*. 2011) and TUDCA (Chen *et al*. 2008, Fang *et al*. 2013) could potentially slow the progression of DN through the attenuation of ER stress-induced apoptosis via the reduction of GRP78 and PERK expression, and the restoration of defective autophagy. However, it is not yet understood whether chemical chaperones could directly improve kidney function or whether these results are simply confounded by the improvement in glycaemic control observed with this class of agents. Despite a recent clinical investigation determining that oral TUDCA administration can increase both hepatic and muscular insulin sensitivities in obese non-diabetic patients (Kars *et al*. 2010), markers of ER stress were not improved and therefore it is evident that there is still relatively little known about the long-term efficacy and target specificity of these ER stress modulators in humans.

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Table 1 ER stress modulators of particular interest

ER stress modulator	Mechanism of action	Cells investigated	Disease model	Outcomes	References
4-Phenyl-butyric acid (4-PBA)	ER chaperone		STZ-induced diabetes	Attenuates proteinuria, oxidative stress markers p-JNK, MCP1 and TGFβ1, ER stress markers GRP78 and PERK	Qi *et al.* (2011)
Taurine-conjugated ursodeoxycholic acid (TUDCA)	ER chaperone	Podocytes		Suppressed AGE-induced elevation of GRP78 and dose-dependently inhibited podocyte apoptosis	Chen *et al.* (2008)
			Clinical trial: obese non-diabetic	Increased hepatic and muscle insulin sensitivities. Markers of ER stress did not change	Kars *et al.* (2010)
Palmitoleic/oleic acid	Attenuated palmitic acid-induced upregulation of CHOP	Podocytes		Reduced palmitic acid-induced apoptosis of podocytes	Sieber *et al.* (2010)
Rapamycin	Specific suppression of IRE1–JNK signalling	PTCs	TM-treated mice	Suppressed renal tubular injury	Kato *et al.* (2012)
		Podocytes	PcKOTsc1 mice	Early intervention regenerated podocyte foot processes Reversed mTORC1-dependent podocyte injury	Inoki *et al.* (2011)

STZ, streptozotocin; TM, tunicamycin.

Rapamycin (sirolimus), an mTOR inhibitor, is currently approved by the FDA as an anti-restenotic agent and immunosuppressant. Clinical studies of rapamycin have shown significant efficacy in improving glycaemic control in T1D patients following pancreatic islet transplantation (Shapiro *et al.* 2000). Moreover, sirolimus-based trials of kidney transplantation also improved acute rejection and minimised nephrotoxicity often observed with other immunomodulators (Kahan *et al.* 1998, Groth *et al.* 1999). However, it is difficult to determine if this class of agent or rapamycin-based therapeutics could be beneficial in the treatment of DN. Although results from mouse-based experimental models indicate that a reduction in mTORC1 activity could reduce the development of DN (Inoki *et al.* 2011). Pharmacological inhibition of the mTOR pathway by rapamycin should be approached cautiously, given the evident off-target effects described with the longstanding application of rapamycin (Sarbassov *et al.* 2006).

Chemical chaperones that enhance protein folding are not the only avenue for ER modulation. Indeed, agents targeting other pathways such as dietary intervention through the reduction of palmitic acid could reduce ER-stress-mediated CHOP upregulation in podocytes and thereby attenuate pro-apoptotic pathways mediated by ER stress (Sieber *et al.* 2010). Moreover, antioxidant therapy using α-lipoic acid has been determined to reduce hepatic lipid accumulation, ER stress biomarkers and glomerular mesangial matrix expansion (Melhem *et al.* 2002, Min *et al.* 2012) in other contexts. There is evidence that increasing consumption of polyunsaturated fatty acids could attenuate DN both in animal models and in humans (Shapiro *et al.* 2011), although it remains to be determined as to whether this is modulated through improvement in ER stability. These results indicate that perhaps dietary intervention involving a shift to increased unsaturated FFAs and reduced saturated fats could reduce ER-stress-related renal cellular toxicity, particularly in T2D where lipid accumulation plays a more integral role in disease pathology.

Concluding remarks

Whether or not ER stress directly contributes to the pathogenesis of nephropathy in the context of diabetes is still an unanswered question, but it is apparent that there is ER stress within specific renal cells. Furthermore, there are certainly lessons to be learnt from other sites within the body, as it is evident that ER stress is not a uniform phenomenon, particularly affecting highly synthetic sites.

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Hence, drawing similarities between seemingly disparate sites which commonly present with ER stress in disease states may assist us to further understand this mystery. However, whether or not ER stress plays a causative role in the pathogenesis of DN is not yet defined, nor is the regulatory role of the UPR in highly synthetic organs such as the kidney well understood. Certainly, using modulators of ER function and stress in experimental models of DN may provide some answers in this context. However, the ability of these modulators to specifically target the renal cells of interest and their mechanism of action need to be further explored particularly given the complexity of the kidney, which contains more than eight resident cell types. There should also be an emphasis placed on the investigation of societal and psychological implications as to how to best integrate these novel therapeutics in conjunction with proven treatment regimens to assist in improving government regulatory authorities, patient compliance and ultimately patient health and well-being.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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