Teaser *Endoplasmic reticulum stress, autophagy, and their interplay are crucial in the development of diabetes and associated microvascular complications, requiring further investigations.*

ER stress response mediates diabetic microvascular complications

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Endoplasmic reticulum (ER) homeostasis orchestrates the folding, modification, and trafficking of secretory and membrane proteins to the Golgi compartment, thus governing cellular functions. Alterations in ER homeostasis result in the activation of signaling pathways, such as the unfolded protein response (UPR), to regain ER homeostasis. Nevertheless, failure of UPR leads to activation of autophagy-mediated cell death. Several recent studies emphasized the association of the ER stress (ERS) response with the initiation and progression of diabetes. In this review, we highlight the contribution of the ERS response, such as UPR and autophagy, in the initiation and progression of diabetes and associated microvascular complications, including diabetic nephropathy (DN), retinopathy, and neuropathy, in various experimental models, as well as in humans. We highlight the ERS as a putative therapeutic target for the treatment of diabetic microvascular complications and, thus, the urgent need for the development of improved synthetic and natural inhibitors of ERS.

Introduction

Diabetes is branded as an epidemic of the current century and, along with its complications, poses a substantial health and economic burden globally. Diabetic complications are categorized into microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications (stroke, peripheral vascular disease, and cardiovascular diseases). Microvascular complications in particular are more prevalent, impacting almost 50% of patients with type 2 diabetes mellitus (T2DM) across 28 countries in Europe, South America, Asia, and Africa [1]. The A1chieve study, an Indian observational study encompassing 20 554 patients with T2DM,

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reported a prevalence of retinopathy, nephropathy, and neuropathy of 32.5%, 30.2%, and 26.8%, respectively [2]. In addition to strict glycemic control, researchers have attempted to target divergent routes leading to diabetic complications, including metabolic, inflammatory, and hemodynamic pathways [3]. However, despite the available therapies, there has been little success in stemming the progression of diabetic complications. Therefore, there is a need to explore novel pathways that might provide a suitable target for therapies that might be useful in preventing the progression of diabetic complications. Over the past few years, ERS and autophagy have emerged as major fields of research to counter the development and progression of diabetes and related complications.

Persistent ERS is linked to both type 1 (T1)DM and T2DM via different mechanisms. Studies showed that using molecular and chemical chaperones alleviated diabetic symptoms and restored glycemic homeostasis, improving in insulin resistance (IR) [4,5]. Reduction of the ERS has been effective in controlling diabetic microvascular complications, including retinopathy, neuropathy, and nephropathy [6–8]. However, chemical chaperones, such as tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyric acid (4-PBA), lack the desired specificity and pharmacological traits to develop them as therapeutic tools. Recently, Suneng *et al*. reported the small molecule azoramide, which acted as both an ERS modulator and an antidiabetic molecule [9]. Although autophagy and ERS are two distinct types of machinery, recent reports reveal that autophagy is an extra layer of protection against accumulated misfolded proteins and acts along with endoplasmic–reticulum-associated protein degradation (ERAD) to clear unfolded and misfolded proteins, thus reducing ERS [10]. Autophagy modulation is also a valuable approach to ameliorate the progression of diabetes and its complications [11–14]. Thus, these reports suggest ERS and autophagy as potential targets in diabetes. Here, we discuss their role in the development of diabetes and microvascular complications.

ERS and UPR

Proteins are the structural and functional units of a cell. Their biological activity depends on their 3D conformational structure acquired after synthesis by ribosomes in the ER [15]. The ER is a voluminous membrane-bound organelle involved in several physiologically vital functions (e.g., synthesis, folding, processing, and transport of proteins, synthesis of lipids, storage and release of Ca^{2+}, and signaling operations) [16,17]. The processes involved from protein synthesis to folding and transport are complex and prone to errors. Improper protein folding can lead to several pathophysiological conditions [18,19]. Interestingly, almost one-third of proteins synthesized by ER under normal physiological conditions are misfolded, suggesting that the situation is worse during pathological states [20]. However, ER maintains the homeostasis between protein manufacture, folding, transport and degradation, a process termed 'proteostasis' [19,21]. An intricate set of mechanisms continually monitors the quality and quantity of proteins synthesized; for example, molecular chaperones assist with the proper folding of proteins as well as the degradation of misfolded proteins [22]. Nevertheless, different environmental and genetic cues along with metabolic alterations can disturb the normal course of protein synthesis and folding, leading to activation of a ubiquitin-proteasome system, macroautophagy, and ERS [23]. The primary response to ERS is activation of UPR, which acts as a sensor monitoring the workload of ER and exerts its actions via different mechanisms to mitigate the accumulated misfolded proteins [24]. UPR alleviates ERS broadly by: (i) a reduction in the load on ER via global inhibition of synthesis of new proteins; (ii) enhanced transcription of constituents of ERAD, which aids the degradation of misfolded proteins; and (iii) enhancing ER activity and size to increase protein synthesis and folding capability [25]. These three major branches of UPR are reported to function in parallel by using a distinct set of signaling pathways. These signal transducers comprise transmembrane proteins, namely protein kinase R/PKR-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) [22].

Under resting conditions, UPR sensors are bound to binding immunoglobulin protein (BiP), which keeps them in an inactive state. However, when unfolded proteins aggregate, the affinity of BiP for UPR sensors decreases and it separates from the sensors, thus activating the UPR signaling pathway [10]. PERK reduces the protein load by inhibiting translation. PERK also acts as an important part of mitochondrial-associated ER membranes (MAMs) and maintenance of mitochondria–ER and plasma membrane–ER contact sites by interacting with Filamin A. PERK is also reported to regulate apoptosis, inflammation, and intracellular Ca^{+2} entry [26]. IRE1α and ATF6 act by inducing the transcription of UPR target genes and chaperones, thus facilitating the removal of accumulated unfolded proteins. IRE1α is also important in the formation of MAMs, which in turn regulate mitochondrial Ca^{+2} uptake [27]. IRE1α also has an important role in facilitating the interaction between ER and mitochondria, thus maintaining cellular bioenergetics [27]. Akin to PERK, IRE1α is also reported to directly interact with filamin A and regulate actin cytoskeleton remodeling [28]. ATF6, the third branch of the UPR, also has an important role in tissue development (osteogenesis, adipogenesis, and neuroembryogenesis), homeostasis, and pathogenesis. Obesity has been linked to the generation of ER stress and is one reason for the development of endothelial dysfunction. Villalobos-Labra *et al*. reported that ATF6 was localized in the nucleus of human umbilical vein endothelial cells (HUVECs) of prepregnancy maternal obese subjects, corroborating the role of obesity in the development of ER stress [29].

UPR is a protective mechanism: it can switch its prosurvival mode to a proapoptotic mode during regular periods of elevated ERS [30]. The UPR pathway is designed to mitigate these acute changes in the proteostasis environment [31]. However, prolonged activation of UPR in chronic conditions, such as diabetes or viral infections, shows that ER stress cannot be reduced. Hence, to protect the system from further damage, the ER elicits multiple apoptotic pathways (e.g., PERK-mediated cell death pathways and IRE1α-mediated cell death pathways) [32].

ERS and diabetes

Several studies point at an active role of pancreatic β cell ERS in the pathophysiology of diabetes [33,34]. Although T1DM and T2DM have different etiologies and different triggers, malfunctioning and longevity of β cells are common to both [35]. T2DM is characterized by persistent hyperglycemia because of the inability of β cells to synthesize insulin or the resistance of different tissues towards insulin [32]. ER is the site for the synthesis, processing, and storage of proinsulin, a precursor of insulin [36]. Insulin is released in the circulation as required. Elevated blood glucose can lead to a steep rise in the translation rate of proinsulin synthesis, which can be as high as ˜20-fold, and almost a 20% of the proinsulin synthesized does not attain its native conformation, indicating that proinsulin is susceptible to misfolding. The high biosynthetic burden on pancreatic β cells compounded with the misfolding-prone nature of proinsulin places the ER of β cells under ERS [36]. This activates UPR signaling pathways to remove the misfolded proinsulin molecules. However, because of persistent hyperglycemia and chronic ERS associated with diabetes, the protective UPR pathways turns destructive for the β cells. Studies support the claim that ERS induced by chronic hyperglycemia and hyperlipidemia leads to the loss of β cell mass and function in T2DM [23]. Among the three UPR sensors, PERK is reported to have an important role in the regulation of β cell development, proliferation, and homeostasis, along with a prominent role in insulin processing and secretion [37]. This is corroborated by studies that showed that PERK-deficient mice exhibited severe β cell malfunctioning and diabetes. Gao *et al*. found that PERK deletion in young as well as adult mice led to structural damage and loss of islet and the β cells, resulting in high glucose levels [38]. In a recent study by Yimeng *et al*., abrogation of the PERK–CCAAT-enhancer-binding protein homologous protein (CHOP) pathway using sodium butyrate led to amelioration of T2DM [39]. PERK was also involved in controlling proinsulin processing by regulating the expression of ER chaperones [40]. PERK was first found in islets of rat pancreas and has an important role in Wolcott–Rallison syndrome (WRS), a rare genetic disorder typified by early onset/neonatal diabetes with a reduction in the mass of β cells not related to autoimmune destruction [41]. In physiological circumstances, IRE1α enhances proinsulin biosynthesis after a meal. However, persistent exposure to high glucose leads to ERS-induced overactivation of the IRE1α signaling pathway, which reduces the expression of insulin genes in β cells [42]. Tsuchiya *et al*. explored the IRE1α–X-box binding protein 1 (XBP1) pathway in maintaining proinsulin and insulin levels along with the oxidative folding of the proinsulin molecules [43]. They found that deficiency of IRE1α in mouse β cells led to the development of diabetes, with the reduced biosynthesis, secretion, and folding of insulin and proinsulin [43].

β Cell homeostasis depends on the balance between the activity of PERK and IRE1α, given that PERK regulates insulin biosynthesis negatively, whereas IRE1α regulates it positively [44]. Any imbalance between the two can be detrimental to β cells. Our understanding of the role of ATF6, the third component of UPR, in diabetes is limited, which warrants further research. ER stress and inflammation crosstalk are central to both T1DM and T2DM pathogenesis. Thus, ER stress is a potential connecting link between inflammation, obesity, and T2DM [45]. T2DM is associated with low-grade inflammation, and ER stress could to the pathogenesis, owing to the proinflammatory state and IR induction. Proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and IL-1β, are common to T2DM and T1DM, and directly hamper the functioning of pancreatic β cells. Different mediators of UPR are influenced by distinct sets of proinflammatory cytokines; for example, IL-1β leads to IRE1α activation, as evidenced by XBP1 splicing and PERK phosphorylation of the α subunit of eIF2 and reduced expression of XBP1 and BiP by IFN-γ [46].

ERS is also actively involved in the development of T1DM, an autoimmune disorder characterized by the destruction of pancreatic β cells [47]. β Cell dysfunction is antecedent to T1DM and ERS is a causative factor of the latter, which can be attributed to activation of the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) pathway [35]. Impairment of UPR components, including ATF6 and XBP1, in the β cells of T1DM mouse models and human samples support the role of ERS and UPR in maintenance of β cells and prevention of T1DM [47]. In addition, the presence of ERS and activation of UPR mediators in pancreatic islets was observed in 13 patients with T1DM [48]. ERS was found to precede insulitis much earlier, forming a microenvironment conducive to the development of autoimmunity and T1DM [49]. It was demonstrated that β cell proteins become immunogenic via Ca^{2+}-regulated post-translational modification when exposed to thapsigargin and physiological triggers of ERS [50]. ERS is a hallmark of T1DM in humans and elevated levels of the proinflammatory cytokines IL-1β, TNF-α, and interferon- alpha (IFN-α) lead to elevation of ER stress, adding to the progression of T1DM [51]. Islet cells isolated from 12 donors without diabetes were exposed to different cytokines, including IFN-α and IL-1β, which resulted in a twofold increase in β cell apoptosis within 24 h [52]. Treatment with TUDCA or knockout of CHOP in these cells led to reduced apoptosis, indicating the involvement of ERS in IFN-α- and IL-1β-mediated apoptosis [52]. Thus, targeting ERS and UPR components has potential as a novel approach to treat diabetes. Examples of ERS modulators and their mechanism of action are provided in Table 1.

Autophagy and diabetes

Autophagy belongs to a class of catabolic processes wherein lysosomes degrade the cytosolic components, including unfolded protein aggregates and injured organelles [53]. Apart from constitutive or basal autophagy, which keeps a check on the quality of the proteins and organelles, the major role of autophagy is to protect cells from stress and starvation by degrading surplus or

TABLE 1

Examples of ERS modulators

ERS modulator	Mode of action	Target Cells/Organs	Refs
TUDCA	ER chaperone	Tubular cells, podocytes	[10,31,73]
4-PBA	ER chaperone	ARPE-19 cells	[8,10,31,105]
Salubrinal	eIF2α phosphatase inhibition	ARPE-19 cells	[10,21,93]
Chrysin	PERK pathway inhibition	Podocytes	[75,92]
Tangluoning	PERK/Nrf2 pathway modulation	Schwann cells (RSC96 cells)	[110]
Quercetin	IRE1α-XBP pathway activation	Liver	[10,21,115]
Guanabenz	eIF2α phosphatase inhibition	HeLa cells	[10,21,116]
Stachydrine	CHOP inhibition	Tubular cells	[117]

worn-out cellular contents and redistributing the nutrients necessary for survival, thus maintaining the cellular homeostasis [54]. Autophagy mediates the physiological functioning of pancreatic β cells along with other tissues, such as liver and skeletal muscles, which are primary targets of insulin action. Pancreatic β cells have a high biosynthetic load and are prone to undergo oxidative ERS, both of which are regulated by autophagy. Autophagy is also involved in the proper functioning of the UPR machinery, and dysfunctional autophagy can impair the UPR, thus making cells prone to the development of ERS [55]. Pancreatic β cell survival requires basal autophagy, given that mice deficient in ATG7 displayed degeneration of islets, reduced insulin release, and glucose tolerance [56]. Overactive autophagy not only augmented insulin signaling by ameliorating ERS, but also reduced insulin secretion, storage, and glucose tolerance [53]. In a study on human islets, Bugliani *et al.* investigated the role of autophagy in β cell functioning and survival [57]. In the study, palmitate (a metabolic ER stressor) exposure led to reduced insulin secretion and elevated β cell apoptosis, which was counteracted by rapamycin (autophagy inducer) treatment and aggravated by 3-methyladenine or concanamycin-A treatment (autophagy inhibitor) [57]. One of the proposed mechanisms by which autophagy might prevent T2DM is breakdown of Notch1, thus promoting β cell neogenesis. Notch1 degradation is associated with increased expression of neurogenin-3, which is a regulator of pancreatic development [58]. A recent report revealed that T2DM aids the repression of autophagy via downregulating the transcription factor EB (TFEB) and lysosomal-associated membrane protein-2 (LAMP-2) [59]. Thus, β cell mass and functioning are heavily dependent upon autophagy flux, and its impairment can lead to diabetes. Several autophagy modulators are in different phases of clinical trial for diseases including Alzheimer's and various types of cancer. However, none have yet advanced to clinical trials for diabetes and only one clinical trial available aims to explore the proautophagy effect of metformin in prediabetic subjects [60]. Thus, efforts should be made to harness the potential of autophagy in the prevention and management of diabetes.

ERS and autophagy crosstalk in diabetes

ERS and autophagy have been observed to regulate each other; for example, ER provides the membrane required in the formation of an autophagosome, given that autophagy-deficient β cells display dysfunctional UPR [61]. In addition, autophagy-related 7 ($Atg7^{\Delta\beta\text{-cell}}$)-ob/ob mice had impaired autophagy [55]. UPR gene expression in β cells was minimal in these mice and autophagy deficiency led to the development of overt diabetes [55]. Bachar-Wikstrom *et al.* observed that suppression of autophagy in β cells from Akita mice led to enhanced ERS and vice versa, along with a finding that defensive action of rapamycin (autophagy activator) was suppressed by chloroquine and bafilomycin A1(autophagy inhibitors), suggesting that persistent ERS leads to the activation of autophagy as a remunerating phenomenon vital for cell survival [62]. Fei-Juan *et al.* found that 4-PBA treatment led to amelioration in diabetic neuronal apoptosis, and *in vitro* treatment with bafilomycin-A1 enhanced neuronal apoptosis, suggesting that autophagy is activated in diabetic conditions as a possible mechanism to prevent neuronal apoptosis via ERS-mediated c-Jun N-terminal kinases (JNK) signaling [63]. ERS-induced autophagy is an important determinant of hepatic physiology and pathophysiology. Quan *et al.* used the OVE26 diabetic mouse model and observed that UPR and autophagy dysfunction during the later stages of diabetes led to the development of steatohepatitis [64]. Obesity and IR are strongly associated with the development of diabetic complications, such as fatty liver [65]. ERS is reported to downregulate the expression of the insulin receptor, hence causing IR [65]. Furthermore, activation of autophagy was observed in distant insulin-sensitive tissues as soon as ERS caused IR [65]. A recent clinical study reported the involvement of the UPR sensor ATF6 in activating proapoptotic signaling along with removal of unfolded and misfolded proteins via autophagy [66]. These findings suggest that autophagy is an adaptive mechanism to alleviate ERS in diabetes. The significance of autophagy is impacts not only the functional aspects of the ER, but also its structural integrity.

ERS and autophagy in diabetic microvascular complications

ERS and autophagy in diabetic nephropathy

The kidneys synthesize almost 42% of total body proteins and have not only a high workload similar to that of pancreatic β cells, but also a high biosynthetic burden of proinsulin synthesis and folding [31]. DN is characterized by thickening of the glomerular basement membrane (GBM) and injury to kidney cells including podocytes along with tubular atrophy. Diabetes further exacerbates the rate of kidney protein synthesis, thus enhancing the probability of ERS and UPR activation [67]. Tubulointerstitial damage is considered to be an important mark of DN progression. Proximal tubular cells form a major chunk of the kidney, with a variety of regulatory and endocrine roles. Proteinuria and hyperglycemia activate tubular cells, leading to the release of proinflammatory mediators and chemokines [68,69]. Tubular cells have a high rate of protein synthesis and are a crucial target of elevated glucose levels, making them prone to ERS [31]. Subsequently, the ERS inhibitor TUDCA ameliorated tubulointerstitial fibrosis in addition to lowering blood glucose level and the urinary albumin:creatinine ratio [70]. Apart from inhibiting ERS, TUDCA also stimulates the farnesoid X receptor, which is abundant in kidney tubular cells and has a protective role in DN [71]. Suppression of ERS markers, such as BiP (GRP78), ATF4, p-PERK, and CHOP, also prevented tubular cell apoptosis in DN [72].

Podocytes are kidney cells crucial in the development of DN given that the differentiated podocytes lack the ability to proliferate and, thus, add to the progression of glomerulosclerosis [31,68]. Podocytes occupy a peculiar position between mesangial cells and tubular cells and have a vital role in regulating the glomerular filtration barrier, with varying permeability for different electrolytes and proteins [68]. Both advanced glycation end products (AGE) and albumin induce ERS and podocyte injury [73,74]. ERS reduction using TUDCA and 4-PBA protected podocytes from apoptosis in DN [8]. Chrysin alleviated podocyte injury via downregulating the expression of the major ERS pathway, PERK–eukaryotic translation initiation factor 2α (eIF2α)–ATF4–CHOP pathway [75]. Reticulon 1 (RTN1) has been implicated in the progression and severity of diabetic kidney disease by inducing ERS, which was confirmed by the renal biopsies from patients with nephropathy, murine models, and HK2 cells [76]. Xiao *et al.* explored the role of RTN1a in tubular cell damage using RTN1a-knockout mice and incubated HK2 cells with human serum albumin. Knockout of RTN1a in HK2 cells as well as animals led to reduced ERS markers and tubular cell apoptosis. Albuminuria, which is a hallmark of DN, leads to tubular damage via expression of RTN1a [77]. The same group of researchers highlighted the role of RTN1a in podocyte injury during DN [78]. Uninephrectomy db/db mice were used to enhance the progression of DN and were treated with TUDCA. Elevated expression of RTN1a and ERS markers, such as GRP78, p-PERK, and CHOP, were observed in the nephropathic mice. However, TUDCA treatment repressed RTN1a and ERS maker expression not only at the protein level, but also at the mRNA level and, thus, stemmed the progression of DN [78].

A high level of basal autophagy was reported to be essential for podocyte survival [79]. Impaired autophagy in podocytes and proximal tubular cells aggravated the progression of DN and restoring the autophagic process arrested the progress of DN [54,80]. Tagawa *et al.* observed that resveratrol successfully protected the podocytes by activating renal autophagy [81]. In the quest to explore novel targets and strategies to enhance autophagy in renal tissues, Kitada *et al.* revealed that a low protein diet in Wistar fatty rats repressed the mammalian target of rapamycin (mTOR) complex-1 signaling pathway, leading to activation of autophagy and resulting in reduced DN [12]. Treatment with exosomes derived from mesenchymal cells significantly enhanced autophagy in renal tissues, as evidenced by the increased number of autophagosomes, microtubule-associated protein 1 light chain 3 (LC3), and Beclin-1, and decreased mTOR, as well as improvements in kidney morphology and fibrotic state [82]. All these beneficial effects were reversed on using 3-methyladenine (3-MA) and chloroquine (autophagy inhibitors), thus confirming the role of autophagy in alleviating DN [82].

ERS and autophagy interplay in the renal tubules was first reported by Kawakami *et al.* using an immortalized rat proximal tubular cell line (IRPTC) [83]. The ERS inducers, Tunicamycin, and brefeldin A, led to the activation of the autophagic process [83]. Fang *et al.* found an interplay between ERS and autophagy in podocytes. Exposure of 3-MA led to enhanced ERS in the podocytes, whereas knockout of CHOP restored the autophagy. This suggests that autophagy aids the removal of unfolded proteins, thus relieving the ER from excess burden, and that inhibition of autophagy led to the accumulation of such proteins, elevating the ERS. Glomerular mesangial cell (GMC) injury is indicative of the progression of DN. GMCs are contractile cells that remove apoptotic cells by phagocytosis to keep the GBM free of debris, as well as secreting mesangial matrix, regulating the glomerular filtration, and providing structural support to the glomerular capillaries [84]. AGE exposure of mesangial cells led to mesangial toxicity, and it is thought to be induced by ERS and autophagic mediated pathways. AGEs were found to trigger autophagy in GMCs by ERS initiation. Autophagy modulation and ERS suppression was effectively mediated by 4-PBA. However, 4-PBA failed to modulate rapamycin-induced autophagy in GMCs, indicating that AGE-induced autophagy was mediated via the eIF2α–CHOP stress pathway [84]. The above-discussed studies demonstrate the existing crosstalk between ERS and autophagy in the initiation and progression of DN, as summarized in Fig. 1.

ERS and diabetic retinopathy

Diabetic retinopathy (DR) is characterized by basement membrane thickening, microaneurysms, pericyte loss, blood–retinal barrier breakdown, acellular capillary, intraretinal microvascular abnormality, neovascularization, and finally retinal detachment [85]. Hyperglycemia allied with reactive oxygen species (ROS), inflammation, and ERS causes damage to the retinal blood vessels as well as neurons [86]. Damage to the blood–retinal barrier is an main feature of DR [87]. Müller cells, a type of crucial glial cell in the retina, are the key cause of inflammation in retinopathy because of their ability to secrete cytokines and various growth factors, such as vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1 (ICAM-1) [88]. Overproduction of VEGF unsettles the blood–retina barrier, leading to vascular exudation and neovascularization [87]. Impairment of the balance of proangiogenic factors, such as VEGF and ICAM-1, and proinflammatory cytokines, such as TNF-α, IL-6, IL-8, and monocyte chemoattractant protein 1 (MCP-1), are crucial to the development of DR [6,87].

Yang *et al.* studied the role of XBP1, a transcription factor and an important component of the UPR, in Müller cells of diabetic mice and primary Müller cells exposed to high glucose and hypoxic conditions [6]. XBP1-deficient diabetic mice had elevated levels of retinal VEGF, TNF-α, and ERS markers, including ATF4, ATF6, CHOP, GRP-78, and eif2α, and these mice showed higher vascular leakage [6]. Similarly, primary Müller cells devoid of XBP1 showed higher ERS and inflammatory markers, which was attenuated by treatment with ERS inhibitors [6]. Impairment of *O*-GlcNAcylation of the retinal Müller cell proteins is another factor involved in the pathogenesis of DR. To evaluate the role of a high-fat diet (HFD)-induced ERS *O*-GlcNAcylation, Dai and colleagues utilized an animal model as well as a rat retinal Müller cell line (TR-MUL cells). Mice fed a HFD for 4 weeks displayed enhanced *O*-GlcNAcylation, which was attributed to elevated ERS [89]. ERS was proposed to enhance Müller cell protein *O*-GlcNAcylation by upregulating the expression of glutamine-fructose-6-phosphate amidotransferase 2 (GFAT2), which in turn increases the flux from the hexosamine biosynthesis pathway (HBP) [89]. ERS induction using thapsigargin and inhibition using chemical chaperones enhanced and repressed, respectively the *O*-GlcNAcylation along with GFAT2 levels in TR-MUL cells, corroborating *in vivo* findings regarding the role of ERS in retinal protein *O*-GlcNAcylation and progression of DR [89]. ATF4, one of the important mediators of UPR, regulated the expression of VEGF and ICAM-1, proinflammatory cytokines vital to the development of DR [90]. In a recent clinical study, aqueous humor and vitreous of patients with peripheral DR were evaluated and significantly higher ATF4 levels were reported [90]. The samples also had elevated levels of proinflammatory mediators, such as IL-6 and MCP-1. A correlation analysis between ERS mediators and inflammatory cytokines revealed a significant correlation between ATF4 and IL-6/MCP-1 levels [90].

AGE might also add to the development of DR by different pathways, including ERS. Recently, De-Wei *et al.* observed enhancement of vascular permeability and leakage in animals treated with Nε-(carboxymethyl) lysine (CML), an AGE product, which was attributed to the tumor progression locus 2 (TPL2)/ATF4/stromal cell-derived factor-1α (SDF1α) axis [91]. The authors found that ATF4 regulated SDF1α expression, which was corroborated in human as well animal serum and aqueous humor samples [91]. Elevated levels of ER stress mediators in retinal pigment epithelium (RPE) cells co-incubated with AGE showed the role of the receptor for advanced glycation end-products (RAGE) in the generation of ERS and consequent damage to the retina in diabetic mice [92]. Furthermore, methylglyoxal led to enhanced apoptosis

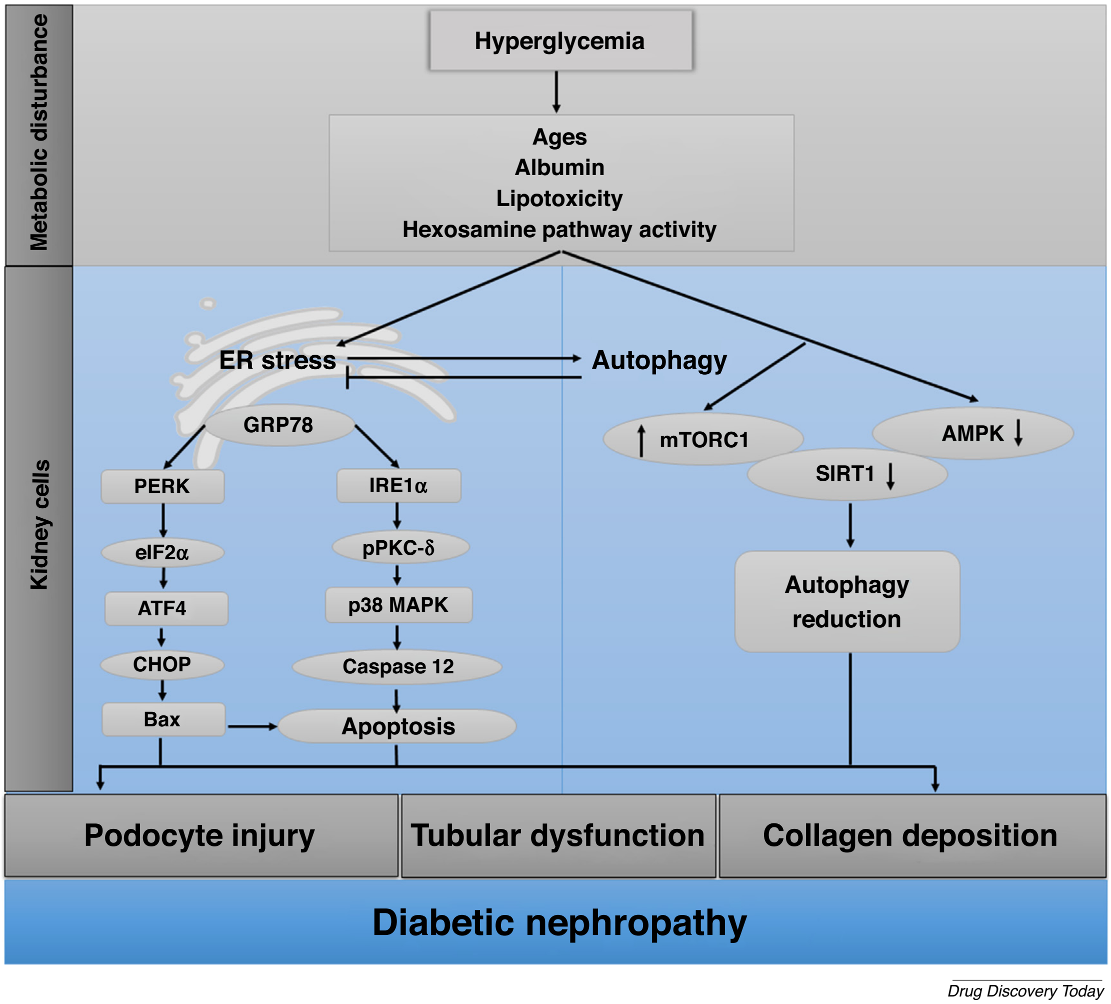

FIGURE 1

An overview of the role of endoplasmic reticulum stress (ERS) and autophagy in the pathogenesis of diabetic nephropathy (CN). Persistent hyperglycemia leads to metabolic disturbances, including AGE formation, lipotoxicity, and albumin excretion, which lead to ERS. Chronic hyperglycemia causes the induction of the proapoptotic unfolded protein response (UPR) by ERS. Activation of the PERK pathway leads to formation of the transcription factor ATF4 and, consequently, CCAAT-enhancer-binding protein homologous protein (CHOP) and Bax, which leads to podocyte and tubular injury. Another proapoptotic pathway of the UPR includes inositol-requiring enzyme 1α (IRE1α), which leads to increased expression of phosphorylated protein kinase C δ (pPKCδ), p38 mitogen-activated protein kinases (p38MAPK), and cleaved caspase 12 expression. These metabolic disturbances also cause suppression of renal autophagy via activation of mammalian target of rapamycin complex 1 (mTORC1) and repression of sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK). ERS also causes suppression of autophagy. All these factors lead to podocyte injury, tubulointerstitial fibrosis, collagen deposition, and finally the development of DN. Abbreviations: AGE, advanced glycation end products; ATF4, activating transcription factor 4; eif2α, eukaryotic initiation factor 2α; GRP78, glucose-regulated protein 78; PERK, protein kinase R-like endoplasmic reticulum kinase.

of adult human RPE cell lines (ARPE-19 cells) via ERS-mediated ROS generation and free intracellular calcium concentration imbalance, which was reversed by treatment with salubrinal and 4-PBA [93]. GRP78 regulates the ERS response via activation of UPR sensors [94]. Elevated levels of GRP78 were correlated with apoptosis of human retinal microvascular endothelial cells (hRECs) and the generation of inflammatory mediators in eyes of patients with DR [94]. Persistent exposure to proinflammatory cytokines in DR is also an important factor in neovascularization. Corroborating the role of GRP78 in vascular angiogenesis, transthyretin (TTR) suppressed the process by elevating GRP78 and facilitating apoptosis of hRECs through the eif2α–CHOP pathway [94]. The role of ERS in the progression of DR is summarized in Fig. 2.

Retina is reported to have abundant expression of autophagy proteins, as shown in a study where rat retinal Müller cells (rMCs) resorted to enhanced autophagy as a prosurvival mechanism when exposed to high glucose. Hyperglycemia led to enhanced autophagy as well as p62/SQTSM1 accumulation because of impairments in the lysosomal machinery. This resulted in VEGF release and apoptosis [13]. In a similar study on rMCs under hyperglycemic conditions, berberine reduced cell death via elevating autophagy and activating AMP-activated protein kinase (AMPK), which also initiated autophagy [95]. Diabetic retinal ganglionic cell apoptosis was significantly increased by inhibition of autophagy using 3-methyladenine (3-MA), corroborating the importance of autophagy in the survival of cells during DR [96]. Diabetic retinas in this case were characterized by increased expression of phosphorylated AMPK and reduced expression of mTOR, both principal regulators of autophagy [96]. Dongxu *et al*. examined the dual role of autophagy in DR. They found

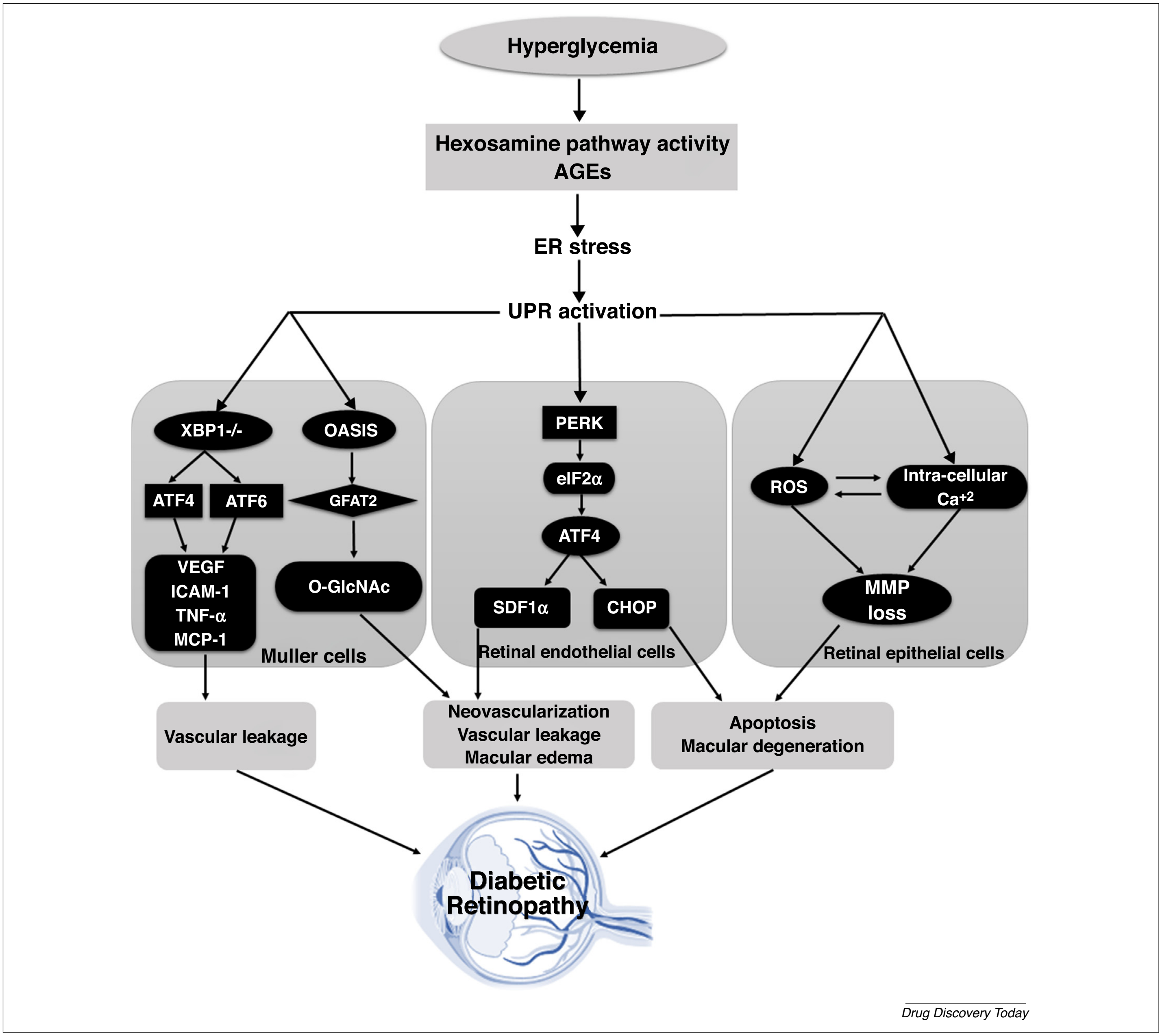

FIGURE 2

A schematic representation of the endoplasmic reticulum stress (ERS) pathways causing damage to cells such as Müller cells, retinal epithelial and retinal endothelial cells, leading to diabetic retinopathy (DR). ERS and the consequent unfolded protein response (UPR) activation can follow different pathways to DR development. X-Box binding protein 1 (XBP1) deficiency can enhance the level of vascular endothelial growth factor (VEGF), intercellular adhesion molecule 1 (ICAM1), tumor necrosis factor α (TNF-α), and MCP-1 via activating transcription factor 4 (ATF4) and ATF6 in Müller cells, causing vascular leakage. During ERS. glutamine-fructose-6-phosphate amidotransferase 2 (GFAT2) binds to old astrocyte specifically-induced substance (OASIS) and increases *O*-GlcNAcylation (*O*-GlcNAc) in the retina, which adds to the progression of DR by initiating neovascularization, vascular leakage, and macular edema. The protein kinase R-like endoplasmic reticulum kinase (PERK)–eukaryotic initiation factor 2α (eif2α)–ATF4–CCAAT-enhancer-binding protein homologous protein (CHOP) pathway and ATF4–stromal cell-derived factor-1α (SDF1α) axis as well as reactive oxygen species (ROS) generation adds to the pathogenesis of DR. Abbreviations: AGE, advanced glycation end products; MCP1, monocyte chemoattractant protein-1. MMP, mitochondrial membrane potential.

that lower concentrations (50 mg/l) of heavily oxidized glycated low-density lipoprotein (HOG-LDL) initiated autophagy-mediated cell survival, whereas higher concentrations (200 mg/l) (i.e., severe stress) led to autophagy-mediated cell death [97]. HOG-LDL also elevated ERS via Jun amino-terminal kinases [97]. Thus, ERS and autophagy might be novel therapeutic targets for DR. Nevertheless, further research is needed to prove their efficacy in combatting DR.

ERS and diabetic neuropathy

Diabetic neuropathies range from acute to chronic and focal to diffused; however, diabetic peripheral neuropathy (DPN) is the most common. Diabetic neuropathy is characterized by increasing pain sensation to tactile stimulation and sensory loss to heat along with paresthesia, hyperalgesia, and allodynia [98]. The histopathological changes include degeneration of peripheral fibers, Schwann cell atrophy, axonal swelling, and demyelination of nerve fiber [99]. Hyperglycemia-induced activation of various pathway, including increased activity of aldose reductase, formation of AGE, activation of protein kinase C, generation of ROS, mitochondrial dysfunction, low-grade inflammation, and reduced blood flow to nerves, are some of the pathogenetic mechanisms associated with diabetic neuropathy [100]. The diabetic milieu also affects astrocytes and microglia, two major types of CNS cell that function to protect and support the neurons [98]. Hyperglycemia and different proinflammatory mediators (IL-1β, TNFα, and IL-6) lead to the activation of microglial cells, which further drives the progression of DPN. This was corroborated by studies that reported elevated levels of activated microglia in experimental diabetic animals and patients with diabetes [98]. Microglial RAGE activation causes release of different chemokines, including CCL3 and CCL5, which in turn activate microglial cells, adding to DPN development [98]. Astrocytes are a primary class of glial cells that are essential for the proper functioning of the central nervous system (CNS) and have vital operations including, but not limited

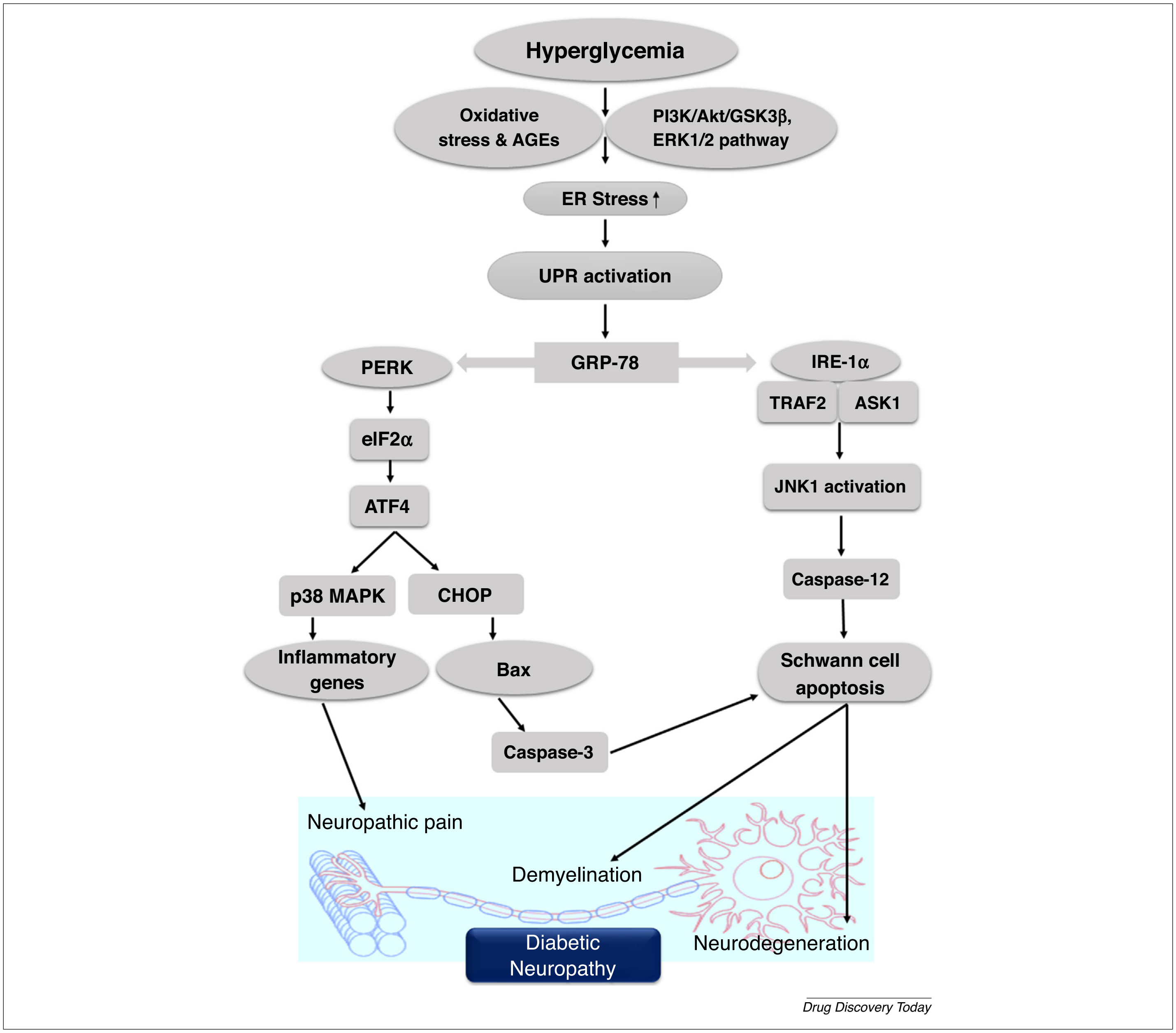

FIGURE 3

Pathogenic role of perpetual endoplasmic reticulum stress (ERS) in the development and progression of diabetic peripheral neuropathy (DPN). Chronic ERS causes activation of caspase-12 of the ER membrane via formation of a complex between inositol-requiring enzyme 1α (IRE1α), TNF receptor-associated factor 2 (TRAF2), and apoptosis signal-regulating kinase 1 (ASK1), which further activates c-Jun N-terminal kinase 1 (JNK), resulting in Schwann cell apoptosis. By contrast, activation of glucose-regulated protein 78 (GRP78) induces the protein kinase R-like endoplasmic reticulum kinase (PERK)–eukaryotic initiation factor 2α (eif2α) pathway. Activating transcription factor 4 (ATF4) is downstream of this pathway and might produce inflammatory genes via p38 mitogen-activated protein kinases (p38MAPK) or initiate apoptosis via CCAAT-enhancer-binding protein homologous protein (CHOP) and Bax expression. All this might finally lead to demyelination, neuronal apoptosis, and development of DPN. Abbreviations: AGE, advanced glycation end-product; PI3K, phosphoinositide 3-kinase; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GSK3β, glycogen synthase kinase 3 beta.

to, homeostasis of fluid, ion, pH, and neurotransmitters, blood flow regulation, and CNS energy metabolism and homeostasis [101]. Yang *et al*. found that oxidative stress and generation of proinflammatory cytokines because of persistent hyperglycemia also activated astrocytes. Hyperglycemia induced the release of ROS from astrocytes, leading to neuronal apoptosis [102]. Furthermore, fluctuation in glucose levels between higher and lower concentrations was also reported to cause astroglial injury [103].

Inceoglu *et al*. revealed the role of ERS and soluble epoxide hydrolase (sEH) regulation of diabetic neuropathy and associated pain. UPR sensors, including PERK, IRE1α, and ATF-6, along with their downstream mediators, were elevated in the peripheral nervous system of T1DM rats [104]. TPPU, an oral inhibitor of sEH when administered concomitantly with 4-PBA, led to a synergistic reduction in neuropathic pain, indicating that ERS is one of the main culprits in the development of neuropathic pain [104]. Tunicamycin, a known ERS inducer, results in enhanced neuropathic pain, again confirming the involvement of ERS in the pathogenesis of neuropathic process [104]. Persistent hyperglycemia disrupts the normal functioning of the nervous system, other than via morphological changes. Sharma *et al*. observed that incubation of dorsal root ganglion (DRG) neurons with high glucose caused increased apoptosis, which was prevented by treatment with 4-PBA, suggesting the involvement of ERS in this process [105]. Schwann cells are another part of the peripheral nervous system, which is highly exposed to damage by hyperglycemia [106]. Rui *et al*. found that rats with DPN had impaired myelin sheaths, nerve fibers that are attributed to excess ERS [107]. When cultured with high glucose *in vitro*, rat Schwann cells (RSC96) exhibited elevated ERS, as evidenced by high GRP78 and CHOP levels [107]. Furthermore, nerve growth factor (NGF) treatment both *in vivo* and *in vitro* led to significant improvement in DPN parameters, which could be ascribed to reduced ERS by NGF [107]. Nevertheless, the short half-life of NGF and its tendency to diffuse swiftly in physiological environments hampers its use in patients with DPN [108]. Rui *et al*. addressed the issue in a preclinical study by preparing a biodegradable coacervate of NGF and basic fibroblast growth factor (bFGF) [108]. They observed that dual delivery of NGF and bFGF significantly alleviated DPN *in vivo* and *in vitro* in RSC96 cells [108]. Furthermore, incubation of Schwann cells with glycolaldehyde induced apoptosis via ERS [109]. Tangluoning, a Chinese herb, has been reported to alleviate ERS in DPN via the PERK/Nrf2 pathway [110]. In a clinical study, patients with DPN showed elevated mRNA expression of CHOP, indicating the potential involvement of CHOP in DPN progression [111]. Yao *et al*. found that IRE1α small interfering (si)RNA transfection inhibited pJNK, caspase 12, and CHOP expression and improved nerve morphology and demyelination in DPN rats [7]. Examples of stimulators of ER stress leading to the development of DPN are shown in Fig. 3.

Protective or deleterious activity of autophagy is still debated in the progression of DPN. Chung *et al*. reported reduced autophagy to be a cause of the development of DPN via decreased AMPK levels [14]. They found that cinacalcet significantly alleviated DPN-related symptoms both in human Schwann cells and sciatic nerve by enhancing autophagy [14]. In addition, salvianolic acid was demonstrated to protect RSC96 cells exposed to hyperglycemic conditions from apoptosis by inhibiting autophagy [112]. By contrast, autophagy activators [e.g., *Lycium barbarum* polysaccharide (LBP) and AMPK] ameliorated diabetes-associated changes in the sciatic nerve of rats [113,114]. Therefore, these studies indicate the vital role of ERS and autophagy in the development of DPN. However, such contrasting results demand further research.

Concluding remarks

Here, we discussed the cytoprotective role of UPR and autophagy in different microvascular complications; however, further in-depth studies are required to establish the mechanisms underlying these processes in different microvascular complications. ERS and autophagy are interlinked phenomena involved in the pathogenesis of diabetes and associated microvascular complications and targeting one could modulate the other, providing a dual benefit. The possible link between ERS and autophagy should also be kept in mind while developing a therapy for targeting either of the two pathways. Despite the amount of research focused on delineating the role of ERS and autophagy in the development and progression of the diabetic microvascular complications, their exact involvement remains elusive. Further research is needed to understand the role of ERS sensors, such as ATF6, in diabetic complications, and the interplay between ERS and autophagy in the alleviation of diabetic microvascular complications, as well as to clarify the role of autophagy inhibition or initiation in promoting cell survival during diabetes. Furthermore, one of the major issues affecting therapies for diabetes and complications is patient compliance because of pill burden. This necessitates the development of novel therapeutics that can target multiple pathways simultaneously, given that diabetes and related complications have multiple etiological origins. Efforts on several fronts are underway to address this issue, one of them being the development of β cell mimetic designer cells. Targeting ERS and autophagy might be a potential answer to this conundrum. Together, ERS, autophagy, and their intricate relationship must be thoroughly investigated, given that they might be potential therapeutic targets for the treatment of diabetes and its associated microvascular complications.

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References

1 Zheng, Y. *et al.* (2018) Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat. Rev. Endocrinol.* 14, 88–98

2 Mohan, V. *et al.* (2013) Current glycemic status and diabetes related complications among type 2 diabetes patients in India: data from the A1chieve study. *J. Assoc. Physicians India* 61 (Suppl. 1), 12–15

3 Sankrityayan, H. *et al.* (2019) Diabetic nephropathy: the regulatory interplay between epigenetics and microRNAs. *Pharmacol. Res.* 141, 574–585

4 Qi, W. *et al.* (2011) Attenuation of diabetic nephropathy in diabetes rats induced by streptozotocin by regulating the endoplasmic reticulum stress inflammatory response. *Metabolism* 60, 594–603

5 Ozcan, U. *et al.* (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 313, 1137–1140

6 Yang, J. *et al.* (2019) Loss of X-box binding protein 1 in Muller cells augments retinal inflammation in a mouse model of diabetes. *Diabetologia* 62, 531–543

7 Yao, W. *et al.* (2018) IRE1α siRNA relieves endoplasmic reticulum stress-induced apoptosis and alleviates diabetic peripheral neuropathy in vivo and in vitro. *Sci. Rep.* 8, 2579
8 Cao, A.L. *et al.* (2016) Ursodeoxycholic acid and 4-phenylbutyrate prevent endoplasmic reticulum stress-induced podocyte apoptosis in diabetic nephropathy. *Lab. Invest.* 96, 610–622
9 Fu, S. *et al.* (2015) Phenotypic assays identify azoramide as a small-molecule modulator of the unfolded protein response with antidiabetic activity. *Sci. Transl. Med.* 7, 292ra298
10 Cybulsky, A.V. (2017) Endoplasmic reticulum stress, the unfolded protein response and autophagy in kidney diseases. *Nat. Rev. Nephrol.* 13, 681–696
11 Lim, H. *et al.* (2018) A novel autophagy enhancer as a therapeutic agent against metabolic syndrome and diabetes. *Nat. Commun.* 9, 1438
12 Kitada, M. *et al.* (2016) A very-low-protein diet ameliorates advanced diabetic nephropathy through autophagy induction by suppression of the mTORC1 pathway in Wistar fatty rats, an animal model of type 2 diabetes and obesity. *Diabetologia* 59, 1307–1317
13 Lopes de Faria, J.M. *et al.* (2016) Defective autophagy in diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 57, 4356–4366
14 Chung, Y.C. *et al.* (2018) Calcimimetic restores diabetic peripheral neuropathy by ameliorating apoptosis and improving autophagy. *Cell Death Dis.* 9, 1163
15 Stolz, A. and Wolf, D.H. (2010) Endoplasmic reticulum associated protein degradation: a chaperone assisted journey to hell. *Biochim. Biophys. Acta* 1803, 694–705
16 Phillips, M.J. and Voeltz, G.K. (2016) Structure and function of ER membrane contact sites with other organelles. *Nat. Rev. Mol. Cell Biol.* 17, 69–82
17 English, A.R. and Voeltz, G.K. (2013) Endoplasmic reticulum structure and interconnections with other organelles. *Cold Spring Harb. Perspect. Biol.* 5, a013227
18 Menzies, F.M. *et al.* (2011) Protein misfolding disorders and macroautophagy. *Curr. Opin. Cell Biol.* 23, 190–197
19 Wang, M. and Kaufman, R.J. (2016) Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature* 529, 326–335
20 Romero, F. and Summer, R. (2017) Protein folding and the challenges of maintaining endoplasmic reticulum proteostasis in idiopathic pulmonary fibrosis. *Ann. Am. Thorac. Soc.* 14 (Suppl. 5), S410–S413
21 Inagi, R. *et al.* (2014) Proteostasis in endoplasmic reticulum—new mechanisms in kidney disease. *Nat. Rev. Nephrol.* 10, 369–378
22 Almanza, A. *et al.* (2018) Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. *FEBS J.* 286, 241–278
23 Salvado, L. *et al.* (2015) Targeting endoplasmic reticulum stress in insulin resistance. *Trends Endocrinol. Metab.* 26, 438–448
24 Lindholm, D. *et al.* (2017) Recent insights into the role of unfolded protein response in ER stress in health and disease. *Front. Cell Dev. Biol.* 5, 48
25 Herbert, T.P. and Laybutt, D.R. (2016) A reevaluation of the role of the unfolded protein response in islet dysfunction: maladaptation or a failure to adapt? *Diabetes* 65, 1472–1480
26 van Vliet, A.R. and Agostinis, P. (2017) PERK and filamin A in actin cytoskeleton remodeling at ER–plasma membrane contact sites. *Mol. Cell. Oncol.* 4, e1340105
27 Carreras-Sureda, A. *et al.* (2019) Non-canonical function of IRE1α determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. *Nat. Cell Biol.* 21, 755–767
28 Urra, H. *et al.* (2018) IRE1alpha governs cytoskeleton remodelling and cell migration through a direct interaction with filamin A. *Nat. Cell Biol.* 20, 942–953
29 Villalobos-Labra, R. *et al.* (2018) Pre-pregnancy maternal obesity associates with endoplasmic reticulum stress in human umbilical vein endothelium. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 3195–3210
30 Walter, P. and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334, 1081–1086
31 Zhuang, A. and Forbes, J.M. (2014) Stress in the kidney is the road to pERdition: is endoplasmic reticulum stress a pathogenic mediator of diabetic nephropathy? *J. Endocrinol.* 222, R97–R111
32 Back, S.H. and Kaufman, R.J. (2012) Endoplasmic reticulum stress and type 2 diabetes. *Annu. Rev. Biochem.* 81, 767–793
33 Clark, A.L. and Urano, F. (2016) Endoplasmic reticulum stress in beta cells and autoimmune diabetes. *Curr. Opin. Immunol.* 43, 60–66
34 Evans-Molina, C. *et al.* (2013) Lost in translation: endoplasmic reticulum stress and the decline of beta-cell health in diabetes mellitus. *Diabetes Obes. Metab.* 15 (Suppl. 3), 159–169
35 Eizirik, D.L. *et al.* (2008) The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr. Rev.* 29, 42–61
36 Sun, J. *et al.* (2015) Proinsulin misfolding and endoplasmic reticulum stress during the development and progression of diabetes. *Mol. Aspects Med.* 42, 105–118
37 Kefalas, G. and Larose, L. (2018) PERK leads a hub dictating pancreatic beta cell homoeostasis. *Biol. Cell* 110, 27–32
38 Gao, Y. *et al.* (2012) PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. *Mol. Cell. Biol.* 32, 5129–5139
39 Hu, Y. *et al.* (2018) Sodium butyrate mitigates type 2 diabetes by inhibiting PERK-CHOP pathway of endoplasmic reticulum stress. *Environ. Toxicol. Pharmacol.* 64, 112–121
40 Sowers, C.R. *et al.* (2018) The protein kinase PERK/EIF2AK3 regulates proinsulin processing not via protein synthesis but by controlling endoplasmic reticulum chaperones. *J. Biol. Chem.* 293, 5134–5149
41 Delepine, M. *et al.* (2000) EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. *Nat. Genet.* 25, 406–409
42 Lipson, K.L. *et al.* (2006) Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab.* 4, 245–254
43 Tsuchiya, Y. *et al.* (2018) IRE1–XBP1 pathway regulates oxidative proinsulin folding in pancreatic β cells. *J. Cell Biol.* 217, 1287–1301
44 Corbett, J.A. (2006) Insulin biosynthesis: the IREny of it all. *Cell Metab.* 4, 175–176
45 Garg, A.D. *et al.* (2012) ER stress-induced inflammation: does it aid or impede disease progression? *Trends Mol. Med.* 18, 589–598
46 Yang, L. *et al.* (2015) Endoplasmic reticulum stress and protein quality control in diabetic cardiomyopathy. *Biochim. Biophys. Acta* 1852, 209–218
47 Engin, F. *et al.* (2013) Restoration of the unfolded protein response in pancreatic beta cells protects mice against type 1 diabetes. *Sci. Transl. Med.* 5 (211), 211ra156
48 Marhfour, I. *et al.* (2012) Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. *Diabetologia* 55, 2417–2420
49 Crookshank, J.A. *et al.* (2018) Changes in insulin, glucagon and ER stress precede immune activation in type 1 diabetes. *J. Endocrinol.* 239, 181–195
50 Marre, M.L. *et al.* (2016) Inherent ER stress in pancreatic islet beta cells causes self-recognition by autoreactive T cells in type 1 diabetes. *J. Autoimmun.* 72, 33–46
51 Engin, F. (2016) ER stress and development of type 1 diabetes. *J. Invest. Med.* 64, 2–6
52 Marroqui, L. *et al.* (2017) Interferon-alpha mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia* 60, 656–667
53 Yamamoto, S. *et al.* (2018) Autophagy differentially regulates insulin production and insulin sensitivity. *Cell Rep.* 23, 3286–3299
54 Tagawa, A. *et al.* (2016) Impaired podocyte autophagy exacerbates proteinuria in diabetic nephropathy. *Diabetes* 65, 755–767
55 Quan, W. *et al.* (2012) Autophagy deficiency in beta cells leads to compromised unfolded protein response and progression from obesity to diabetes in mice. *Diabetologia* 55, 392–403
56 Ebato, C. *et al.* (2008) Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. *Cell Metab.* 8, 325–332
57 Bugliani, M. *et al.* (2019) Modulation of autophagy influences the function and survival of human pancreatic beta cells under endoplasmic reticulum stress conditions and in type 2 diabetes. *Front. Endocrinol.* 10, 1–10 52–52
58 DiNicolantonio, J.J. and McCarty, M. (2019) Autophagy-induced degradation of Notch1, achieved through intermittent fasting, may promote beta cell neogenesis: implications for reversal of type 2 diabetes. *Open Heart* 6, e001028
59 Ji, J. *et al.* (2019) Type 2 diabetes is associated with suppression of autophagy and lipid accumulation in beta-cells. *J. Cell. Mol. Med.* 23, 2890–2900
60 University of New Mexico. A Double-blind, placebo-controlled trial of anti-aging, pro-autophagy effects of metformin in adults with prediabetes (NCT03309007) Available from: https://clinicaltrials.gov/ct2/show/NCT03309007 [Accessed 1 August 2019].
61 Tooze, S.A. and Yoshimori, T. (2010) The origin of the autophagosomal membrane. *Nat. Cell Biol.* 12, 831
62 Bachar-Wikstrom, E. *et al.* (2013) Stimulation of autophagy improves endoplasmic reticulum stress-induced diabetes. *Diabetes* 62, 1227–1237
63 Kong, F.J. *et al.* (2018) Endoplasmic reticulum stress/autophagy pathway is involved in diabetes-induced neuronal apoptosis and cognitive decline in mice. *Clin. Sci.* 132, 111–125
64 Zhang, Q. *et al.* (2015) ER stress and autophagy dysfunction contribute to fatty liver in diabetic mice. *Int. J. Biol. Sci.* 11, 559–568
65 Zhang, N. *et al.* (2015) Autophagy regulates insulin resistance following endoplasmic reticulum stress in diabetes. *J. Physiol. Biochem.* 71, 319–327
66 Diaz-Morales, N. *et al.* (2018) Does metformin modulate endoplasmic reticulum stress and autophagy in type 2 diabetic peripheral blood mononuclear cells? *Antioxid. Redox Signal.* 28, 1562–1569
67 Cunard, R. (2015) Endoplasmic reticulum stress in the diabetic kidney, the good, the bad and the ugly. *J. Clin. Med.* 4, 715–740

68 Leung, J.C.K. *et al.* (2014) Crosstalk between podocytes and tubular epithelial cells. *Podocytopathy* 183, 54–63
69 Tang, S.C. and Lai, K.N. (2012) The pathogenic role of the renal proximal tubular cell in diabetic nephropathy. *Nephrol. Dial. Transplant.* 27, 3049–3056
70 Zhang, J. *et al.* (2016) Tauroursodeoxycholic acid attenuates renal tubular injury in a mouse model of type 2 diabetes. *Nutrients* 8, 589
71 Marquardt, A. *et al.* (2017) Farnesoid X receptor agonism protects against diabetic tubulopathy: potential add-on therapy for diabetic nephropathy. *J. Am. Soc. Nephrol.* 28, 3182–3189
72 Ju, Y. *et al.* (2019) Protective effects of Astragaloside IV on endoplasmic reticulum stress-induced renal tubular epithelial cells apoptosis in type 2 diabetic nephropathy rats. *Biomed. Pharmacother.* 109, 84–92
73 Chen, Y. *et al.* (2008) Effect of taurine-conjugated ursodeoxycholic acid on endoplasmic reticulum stress and apoptosis induced by advanced glycation end products in cultured mouse podocytes. *Am. J. Nephrol.* 28, 1014–1022
74 Gonçalves, G.L. *et al.* (2018) Intracellular albumin overload elicits endoplasmic reticulum stress and PKC-delta/p38 MAPK pathway activation to induce podocyte apoptosis. *Sci. Rep.* 8, 18012
75 Kang, M.K. *et al.* (2017) Chrysin ameliorates podocyte injury and slit diaphragm protein loss via inhibition of the PERK-eIF2alpha-ATF-CHOP pathway in diabetic mice. *Acta Pharmacol. Sin.* 38, 1129–1140
76 Fan, Y. *et al.* (2015) RTN1 mediates progression of kidney disease by inducing ER stress. *Nat. Commun.* 6, 7841
77 Xiao, W. *et al.* (2016) Knockdown of RTN1A attenuates ER stress and kidney injury in albumin overload-induced nephropathy. *Am. J. Physiol. Renal Physiol.* 310, F409–F415
78 Fan, Y. *et al.* (2017) Rtn1a-mediated endoplasmic reticulum stress in podocyte injury and diabetic nephropathy. *Sci. Rep.* 7, 323
79 Fang, L. *et al.* (2013) Autophagy attenuates diabetic glomerular damage through protection of hyperglycemia-induced podocyte injury. *PLoS One* 8, e60546
80 Xu, Y. *et al.* (2015) The renoprotective role of autophagy activation in proximal tubular epithelial cells in diabetic nephropathy. *J. Diabetes Complications* 29, 976–983
81 Huang, S.S. *et al.* (2017) Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of diabetic nephropathy. *Sci. Rep.* 7, 45692
82 Ebrahim, N. *et al.* (2018) Mesenchymal stem cell-derived exosomes ameliorated diabetic nephropathy by autophagy induction through the mTOR signaling pathway. *Cells* 7, E226
83 Kawakami, T. *et al.* (2009) Endoplasmic reticulum stress induces autophagy in renal proximal tubular cells. *Nephrol. Dial. Transplant.* 24, 2665–2672
84 Chiang, C.K. *et al.* (2016) Involvement of endoplasmic reticulum stress, autophagy, and apoptosis in advanced glycation end products-induced glomerular mesangial cell injury. *Sci. Rep.* 6, 34167
85 Cai, J. and Boulton, M. (2002) The pathogenesis of diabetic retinopathy: old concepts and new questions. *Eye* 16, 242–260
86 Volpe, C.M.O. *et al.* (2018) Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis.* 9, 119
87 Wong, T.Y. *et al.* (2016) Diabetic retinopathy. *Nat. Rev. Dis. Primers* 2, 16012
88 Zhong, Y. *et al.* (2012) Activation of endoplasmic reticulum stress by hyperglycemia is essential for Muller cell-derived inflammatory cytokine production in diabetes. *Diabetes* 61, 492–504
89 Dai, W. *et al.* (2018) High-fat diet/palmitate-induced ER stress promotes protein O-GlcNAcylation in retina and retinal Muller cells. *Diabetes* 67 (Suppl. 1), 607
90 Wang, Y. *et al.* (2017) Elevated activating transcription factor 4 and glucose-regulated 78 Kda protein levels correlate with inflammatory cytokines in the aqueous humor and vitreous of proliferative diabetic retinopathy. *Curr. Eye Res.* 42, 1202–1208
91 Lai, D.W. *et al.* (2017) TPL2 (therapeutic targeting tumor progression locus-2)/ATF4 (activating transcription factor-4)/SDF1alpha (chemokine stromal cell-derived factor-alpha) axis suppresses diabetic retinopathy. *Circ. Res.* 121, e37–e52
92 Kang, M.K. *et al.* (2018) Chrysin ameliorates malfunction of retinoid visual cycle through blocking activation of AGE-RAGE-ER stress in glucose-stimulated retinal pigment epithelial cells and diabetic eyes. *Nutrients* 10, E1046
93 Chan, C.M. *et al.* (2016) Methylglyoxal induces cell death through endoplasmic reticulum stress-associated ROS production and mitochondrial dysfunction. *J. Cell. Mol. Med.* 20, 1749–1760
94 Shao, J. *et al.* (2017) Transthyretin exerts pro-apoptotic effects in human retinal microvascular endothelial cells through a GRP78-dependent pathway in diabetic retinopathy. *Cell. Physiol. Biochem.* 43, 788–800
95 Chen, H. *et al.* (2018) Berberine attenuates apoptosis in rat retinal Muller cells stimulated with high glucose via enhancing autophagy and the AMPK/mTOR signaling. *Biomed. Pharmacother.* 108, 1201–1207
96 Park, H.–Y.L. *et al.* (2018) Different contributions of autophagy to retinal ganglion cell death in the diabetic and glaucomatous retinas. *Sci. Rep.* 8, 13321
97 Fu, D. *et al.* (2016) Survival or death: a dual role for autophagy in stress-induced pericyte loss in diabetic retinopathy. *Diabetologia* 59, 2251–2261
98 Rajchgot, T. *et al.* (2019) Neurons and microglia; a sickly-sweet duo in diabetic pain neuropathy. *Front. Neurosci.* 13, 25
99 Sango, K. *et al.* (2017) Impaired axonal regeneration in diabetes. Perspective on the underlying mechanism from in vivo and in vitro experimental studies. *Front. Endocrinol.* 8, 1–8 12–12
100 Grisold, A. *et al.* (2017) Mediators of diabetic neuropathy: is hyperglycemia the only culprit? *Curr. Opin. Endocrinol. Diabetes Obes.* 24, 103–111
101 Sofroniew, M.V. and Vinters, H.V. (2010) Astrocytes: biology and pathology. *Acta Neuropathol.* 119, 7–35
102 Yang, C.M. *et al.* (2017) High-glucose-derived oxidative stress-dependent heme oxygenase-1 expression from astrocytes contributes to the neuronal apoptosis. *Mol. Neurobiol.* 54, 470–483
103 Quincozes-Santos, A. *et al.* (2017) Fluctuations in glucose levels induce glial toxicity with glutamatergic, oxidative and inflammatory implications. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863, 1–14
104 Inceoglu, B. *et al.* (2015) Endoplasmic reticulum stress in the peripheral nervous system is a significant driver of neuropathic pain. *Proc. Natl. Acad. Sci. U. S. A.* 112, 9082–9087
105 Sharma, D. *et al.* (2016) Effects of 4-phenyl butyric acid on high glucose-induced alterations in dorsal root ganglion neurons. *Neurosci. Lett.* 635, 83–89
106 Hao, W. *et al.* (2015) Hyperglycemia promotes Schwann cell de-differentiation and de-myelination via sorbitol accumulation and Igf1 protein down-regulation. *J. Biol. Chem.* 290, 17106–17115
107 Li, R. *et al.* (2017) NGF attenuates high glucose-induced ER stress, preventing Schwann cell apoptosis by activating the PI3K/Akt/GSK3beta and ERK1/2 pathways. *Neurochem. Res.* 42, 3005–3018
108 Li, R. *et al.* (2017) Dual delivery of NGF and bFGF coacervater ameliorates diabetic peripheral neuropathy via inhibiting Schwann cells apoptosis. *Int. J. Biol. Sci.* 13, 640–651
109 Sato, K. *et al.* (2015) Glycolaldehyde induces endoplasmic reticulum stress and apoptosis in Schwann cells. *Toxicol. Rep.* 2, 1454–1462
110 Yang, X. *et al.* (2017) Tangluoning, a traditional Chinese medicine, attenuates in vivo and in vitro diabetic peripheral neuropathy through modulation of PERK/Nrf2 pathway. *Sci. Rep.* 7, 1014
111 El-Horany, H.E.-S. *et al.* (2019) Expression of LRP1 and CHOP genes associated with peripheral neuropathy in type 2 diabetes mellitus: correlations with nerve conduction studies. *Gene* 702, 114–122
112 Wang, Q.-Q. *et al.* (2019) Salvianolic acid B inhibits the development of diabetic peripheral neuropathy by suppressing autophagy and apoptosis. *J. Pharm. Pharmacol.* 71, 417–428
113 Liu, S.Y. *et al.* (2018) Lycium barbarum polysaccharide protects diabetic peripheral neuropathy by enhancing autophagy via mTOR/p70S6K inhibition in streptozotocin-induced diabetic rats. *J. Chem. Neuroanat.* 89, 37–42
114 Yerra, V.G. *et al.* (2017) Adenosine monophosphate-activated protein kinase abates hyperglycaemia-induced neuronal injury in experimental models of diabetic neuropathy: effects on mitochondrial biogenesis, autophagy and neuroinflammation. *Mol. Neurobiol.* 54, 2301–2312
115 Liu, C.M. *et al.* (2013) Protective effect of quercetin on lead-induced oxidative stress and endoplasmic reticulum stress in rat liver via the IRE1/JNK and PI3K/Akt pathway. *Free Radic. Res.* 47, 192–201
116 Tsaytler, P. *et al.* (2011) Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* 332, 91–94
117 Zhang, C. *et al.* (2013) Effect of stachydrine on endoplasmic reticulum stress-induced apoptosis in rat kidney after unilateral ureteral obstruction. *J. Asian Nat. Prod. Res.* 15, 373–381