Diabetic retinopathy, a common complication of diabetes, is the leading cause of blindness in adults. Diabetes chronically damages retinal blood vessels and neurons likely through multiple pathogenic pathways such as oxidative stress, inflammation, and endoplasmic reticulum (ER) stress. To relieve ER stress, the cell activates an adaptive mechanism known as the unfolded protein response (UPR). The UPR coordinates the processes of protein synthesis, protein folding, and degradation to ensure proteostasis, which is vital for cell survival and activity. Emerging evidence suggests that diabetes can activate all three UPR branches in retinal cells, among which the PERK/ATF4 pathway is the most extensively studied in the development of diabetic retinopathy. X-box binding protein 1 (XBP1) is a major transcription factor in the core UPR pathway and also regulates a variety of genes involved in cellular metabolism, redox state, autophagy, inflammation, cell survival, and vascular function. The exact function and implication of XBP1 in the pathogenesis of diabetic retinopathy remain elusive. Focusing on this less studied pathway, we summarize recent progress in studies of the UPR pertaining to diabetic changes in retinal vasculature and neurons, highlighting the perspective of XBP1 as a potential therapeutic target in diabetic retinopathy.

1. Introduction

Over the last two decades, diabetes mellitus (DM) has become one of the most challenging health problems worldwide. It is estimated that there were 366 million people with diabetes in 2011, and the number is expected to rise to 552 million by 2030 [1]. Potential causes for this rapid growth in the diabetic population include aging, urbanization, and increasing prevalence of obesity and physical inactivity [2]. According to The Eye Diseases Prevalence Research Group, the estimated crude prevalence rates of diabetic retinopathy (DR) and vision-threatening retinopathy are as much as 40.3% and 8.2%, respectively, in American adults 40 years and older known to have DM [3, 4]. Half of the patients with untreated proliferative retinopathy will lose their sight within five years [5]. Although good glycemic control has been shown to significantly reduce the incidence of microvascular complication, unfortunately only 17% of patients could achieve the goal of reducing glycated hemoglobin (HbA1C) to levels less than 7% [6]. Even with intensive metabolic control, up to 20% of patients will develop proliferative diabetic retinopathy (PDR) after having diabetes for 30 years [6]. In developing countries such as China and India, the prevalence of diabetes is increasing at an alarming rate [1]. Lack of a sound healthcare system and insufficient public awareness of the disease, together with uncontrolled hyperglycemia, hypertension, and hyperlipidemia, have imposed a much higher risk for the incidence of severe diabetic complications in these countries. Therefore, understanding the pathogenesis of diabetic damage, while developing novel and effective therapies to prevent diabetes-related vision loss is significant unmet needs.

In recent years, studies have demonstrated that dysfunction of the endoplasmic reticulum (ER), or ER stress, is involved in the pathogenesis of diabetes and its complications [7, 8]. The ER is the central cellular organelle responsible for protein folding and maturation. To ensure the fidelity of protein folding, the ER possesses sophisticated machinery...
to recognize aberrant proteins and target them for refolding or clearance [9]. This process is known as the unfolded protein response (UPR). Through comprehensive approaches that regulate ER-associated protein degradation (ERAD), protein translation, and protein folding, the UPR works toward diminishing ER stress and restoring ER homeostasis. However, when prolonged ER stress exceeds the ability of the UPR, ER homeostasis cannot be reestablished. Under these conditions, the UPR will switch from adaptive to proapoptotic actions that induce cell death to remove severely stressed and unhealthy cells (reviewed in [10]). Therefore, the central role of the UPR in human disease involves a life-or-death decision, according to an assessment of whether ER stress can be relieved in a timely fashion [11]. To date, there are three UPR branches that have been identified, namely, the inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6), and protein kinase RNA- (PKR-) like ER kinase (PERK) pathways. In recent years, studies have provided experimental evidence that all three UPR branches are activated in DR, contributing to retinal inflammation, apoptosis, and angiogenesis [12–18]. Among these UPR branches, the PERK pathway and its downstream target ATF4 have been thoroughly studied in retinal cells in DR, whilst the roles of the IRE1 and ATF6 pathways remain largely unexplored. X-box binding protein 1 (XBP1) is the transcription factor activated by IRE1 during ER stress. Activation of XBP1 is critical for maintaining ER function and dysregulated XBP1 expression/activity has been linked to apoptosis, cell death, and insulin resistance in diseased conditions [19]. Deletion or downregulation of XBP1 increases retinal cell sensitivity to apoptosis [20], inflammation [12], and oxidative stress [21]. However, direct evidence of XBP1 in the development of DR is still lacking. In this review, we will focus on the XBP1 pathway and provide a perspective regarding XBP1 as a potential target in DR.

2. Activation of the UPR Branches

The UPR pathways are activated by three ER stress sensors located on the ER membrane, namely, IRE1, ATF6, and PERK. In resting cells, the ER luminal domain of these proteins binds to a chaperone molecule called the glucose-regulated protein 78 (GRP78, also known as immunoglobulin binding protein, BiP) and the binding prevents their activation. Upon ER stress, GRP78 is sequestered from the sensors and binds to unfolded/misfolded proteins to facilitate their refolding. The dissociation of GRP78 results in activation of the ER stress sensors and subsequently activates the UPR [19]. The three UPR pathways can be activated differentially and independent of each other and play distinct roles in inducing downstream target genes and regulating various physiological processes (Figure 1).

2.1. The IRE1/XBP1 Pathway. This pathway is the conserved core of UPR, because of its similarity from yeast to humans [19]. There are two different IRE1 in mammalian cells, IRE1α and IRE1β. IRE1α is ubiquitously expressed, while IRE1β is tissue specific [22, 23]. IRE1 is a bifunctional protein acting as a transmembrane kinase and endogenous ribonuclease (RNase) [24, 25]. When activated, IRE1 autophosphorylates and splices a 26-nucleotide intron from the mRNA encoding a UPR-specific transcriptional factor called XBPI, which results in a frame shift of the XBPI gene [26]. The frame shift gives rise to spliced XBPI or XBPIs, which is a more stable and potent activator of UPR genes [27]. XBPIs induces downstream genes, including ER chaperones and proteins involved in ERAD, and these proteins are thought to play a central role in restoring ER homeostasis and promoting cell survival [28–30].

2.2. The PERK/eIF2α/ATF4 Pathway. PERK superficially resembles IRE1, which is activated by autophosphorylation, and then phosphorylates the α-subunit of eukaryotic translation factor-2 (eIF2) at Ser51 with its cytoplasmic protein kinase domain [31]. This phosphorylation inhibits the reactivation of eIF2 into its GTP-bound form, resulting in global arrest of protein synthesis that, in turn, prevents further influx of ER client proteins [19, 31]. In addition to reducing translation, PERK-mediated phosphorylation of eIF2α also induces preferential translation of activating transcription factor 4 (ATF4), a transcription activator of the integrated stress response [32]. ATF4 induces a subset of ER stress genes involved in inflammation, apoptosis, and oxidative stress by binding to the cAMP-response element (CRE) located in their promoter or enhancer regions [33]. Two representative genes driven by ATF4 are C/EBP homologous protein (CHOP) and growth arrest and DAN-damage-inducible 34 (GADD34). CHOP is a transcription factor regulating the genes encoding components involved in apoptosis [34], while GADD34 encodes a PERK-inducible regulatory subunit of the protein phosphatase PPIC that counteracts PERK by dephosphorylating eIF2α [35]. Recent studies show that the PERK/p-eIF2α/ATF4 pathway is required not only for translational control, but also for activation of ATF6 and its target genes. The PERK pathway facilitates both the synthesis of ATF6 and trafficking of ATF6 from the ER to the Golgi for intramembrane proteolysis and activation of ATF6 [36].

2.3. The ATF6 Pathway. ATF6 has been identified as a basic leucine zipper (bZIP) containing transcription factor that behaves differently than the other two sensors of UPR, as it is packaged into transport vesicles and trafficked to the Golgi apparatus instead of remaining in the ER and acting via cytoplasmic effectors [37, 38]. It is then cleaved by site 1 and site 2 proteases that subsequently remove the transmembrane and luminal domains [39]. Cleaved ATF6 moves to the nucleus and binds to the promoter of ER stress response element related genes encoding ER chaperones and enzymes, such as GRP78, GRP94, and protein disulfide isomerase (PDI) [38, 40, 41]. ATF6 also regulates the transcription of other UPR targets including XBPI [27] and CHOP [42] as well as genes involved in ER-associated degradation [19, 43, 44].

3. The UPR and Diabetic Retinopathy

Clinical features of DR first detected in the 19th century by ophthalmoscopy include retinal vascular abnormalities, such
Figure 1: UPR signaling pathways in retinal cell physiology. Three ER stress sensors PERK, IRE1, and ATF6 are activated when unfolded proteins tether the ER chaperone BiP/GRP78 away from the sensors. Upon activation, IRE1 autophosphorylates and forms a dimer or oligomer that mediates the unconventional splicing of XBP1 mRNA. This process generates the active transcription factor XBP1s that regulates genes involved in ER-associated proteindegradation (ERAD), autophagy, ER quality control, and redox homeostasis. Activated IRE1 recruits TRAF2 and induces activation of JNK and NF-κB leading to increase transcription of inflammatory genes. Once removed from BiP/GRP78, AFT6 moves to the Golgi where it is cleaved by local site 1 and site 2 protease. The cleaved ATF6 is a transcription factor that regulates a subset of components for ERAD and ER chaperones. Like IRE1, PERK is activated by autophosphorylation and phosphorylates the translation initiation factor eIF2α, which suppresses the general translation but selectively increases the translation of ATF4. ATF4 subsequently upregulates the proapoptotic gene CHOP and also modulates the transcription of a variety of genes in inflammation, oxidative stress, apoptosis, and stress responses.
Table 1: Implications of UPR pathways in retinal pathophysiology pertinent to DR.

| Branch of UPR | Cell type | Function | Involved mechanism(s) | References |
|---------------|-----------|----------|-----------------------|------------|
| IRE1/XBP1     | MRPE cells, ARPE-19 cells | Apoptosis | Oxidative stress and Bcl-2 signaling | [21, 122] |
| IRE1/XBP1     | HRECs     | Hypoxia- or TNF-α-induced inflammation | TNF-α/IKK/IKB/NF-κB, and VEGF | [12, 51] |
| IRE1/XBP1     | BREC     | VEGF- or high glucose-induced angiogenesis | VEGF degradation | [59] |
| IRE1/XBP1     | ARPE19 cells | Proliferation | Not mentioned | [123] |
| IRE1/XBP1     | ARPE19 cells | Inflammation and angiogenesis | VEGF transcriptional regulation | [124] |
| IRE1/XBP1     | rMC-1 | Inflammation and apoptosis | Oxidative stress, inflammasome, and autophagy | [74] |
| IRE1/XBP1     | RGCs     | Ischemia-, NMDA-, or IOP-induced apoptosis | Rat model, ASK1, SEK1, and pJNK | [68, 70] |
| ATF6          | BREC     | Angiogenesis | VEGF degradation | [59] |
| ATF6          | MIO-M1   | Apoptosis | Oxidative stress | [73] |
| PERK/ATF4/CHOP | HRECs, TR-iBRB | Inflammation | TNF-α, ICAM-1, VEGF, STAT3 signaling | [12, 18] |
| PERK/ATF4/CHOP | rMC-1 | High glucose-induced inflammation | JNK signaling, HIF-1α/VEGF | [15] |
| PERK/ATF4/CHOP | HRP     | High glucose-induced inflammation | MCP-1 and VEGF | [53] |
| PERK/ATF4/CHOP | ARPE-19 cells | Apoptosis | Oxidative stress and mitochondrial dysfunction mediate apoptosis | [122] |
| PERK/ATF4     | ARPE-19 cells | Inflammation and apoptosis | VEGF transcriptional regulation and oxidative stress | [65, 122, 125] |
| PERK/ATF4/CHOP | HRP     | Apoptosis | Oxidative stress, mitochondrial dysfunction, and autophagy | [16, 52] |
| PERK/CHOP     | hTERT-RPE | High glucose-induced apoptosis | Oxidative stress, mitochondrial dysfunction, and autophagy | [17] |
| PERK/CHOP     | MIO-M1   | Apoptosis | Oxidative stress | [73] |
| PERK/CHOP     | RGCs, RGC-5 | NMDA-, IOP-, or diabetes/high glucose-induced apoptosis | Neurotrophin-4 | [69–71, 126] |

HRECs: human retinal endothelial cells; HUVECs: human umbilical vein endothelial cells; HARC: human aortic endothelial cells; BREC: bovine retinal endothelial cells; TR-iBRB: immortalized rat retinal capillary endothelial cells; rMC-1: rat retinal Müller cells; HRP: primary human retinal pericytes; MIO-M1: human retinal Müller cells; RGCs: retinal ganglion cells; RGC-5: a rat ganglion cell line transformed with EIA virus; MRPE cells: primary mouse RPE cells; ARPE-19: an immortalized human RPE cell line; hTERT-RPE: RPE cells immortalized with hTERT.

As microaneurysm, intraretinal hemorrhages, and cotton-wool spots [45]. With disease progression, loss of blood vessels results in retinal ischemia, which may be accompanied by venous beading, loops, and intraretinal microvascular abnormalities (IRMA). These changes eventually lead to retinal neovascularization (a hallmark of proliferative diabetic retinopathy), fibrosis, and retinal detachment. Breakdown of the blood-retinal barrier (BRB) is another important feature of DR, which can occur at any stage of the disease, advancing from macular edema and exudation in the nonproliferative phase to highly permeable neovascularization in the proliferative phase [46]. Although BRB damage primarily involves disturbed function of tight junctions between vascular endothelial cells (inner BRB) and retinal pigment epithelial cells (outer BRB), emerging evidence suggests that other neural and vascular cells (e.g., glial cells and pericytes), play a role in maintaining normal BRB function [47]. Neuronal and glial cells release metabolites, such as lactate and nitric oxide (NO), which regulate retinal blood flow to meet the metabolic needs of retinal tissue. Alterations in the interactions between neural and vascular cells contribute to vascular dysfunction in the pathogenesis of DR [45]. Although the mechanisms underlying the neurovascular dysfunction in diabetes are not fully understood, recent studies have provided some encouraging evidence suggesting a role of the UPR in DR-related retinal pathophysiology (Table 1).

3.1. The UPR, Inflammation, and Vascular Leakage. Recent studies have revealed that inflammation plays a causal role in diabetes-induced retinal vascular leakage. Accumulation of serum components from leaking blood vessels causes thickening of the fovea or macular edema. Diabetic retina also expresses high levels of proinflammatory cytokines, such as vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), and intercellular adhesion molecular (ICAM)-1, which increase leukocyte (mainly monocyte/macrophage)-endothelial cell interaction and disrupt tight junctions leading to vascular leakage...
ER stress in pericytes [52]. These results suggest that unsta-
fying that glucosedeprivation but nothigh glucose causes
glycated low-density lipoprotein (LDL) activates oxidative
Fu and associates demonstrated that heavily oxidized and
(EC-SOD), an antioxidant enzyme that protects cells from
damage. Among many etiological factors, oxidative stress
is a key insult responsible for vascular pathology in DR.
However, signaling pathways of the UPR branches and reciprocal regulations between oxidative stress
and ER stress, alterations in the ER and mitochondrial
dysfunction, the UPR, and autophagy in diabetes-related
pericyte damage remain to be investigated.

3.3. UPR and Angiogenesis. Retinal angiogenesis (neovascu-
larization, NV), arising on the optic nerve and at the junction
of nonperfused and perfused areas, is the hallmark of PDR,
the advanced stage of DR. Untreated neovascularization leads
to vitreous hemorrhage and tractional retinal detachment
resulting in sudden or permanent vision loss in diabetic
patients. Although the short-term effect of newly developed
anti-VEGF therapy is encouraging, a large percentage of
patients do not respond favorably to these therapies,
suggesting there are unknown mechanisms that regulate retinal
NV in diabetes (reviewed in [55]). While the UPR has long
been recognized as an important mechanism for cancer
cell survival, activation of the UPR by ER stress has also
been shown to contribute to tumor angiogenesis [56]. As an
example, the ER chaperone GRP78 plays an essential role
in tumor proliferation, survival, and angiogenesis in tumor
development [56]. Research from our lab demonstrated that
ER stress is induced in ischemic retina and contributes
to retinal NV formation in oxygen-induced retinopathy
(OIR) [12]. Excessive ER stress induces VEGF in human
retinal endothelial cells [57], while inhibiting ER stress by
chemical chaperone 4-phenobutyrate successfully reduces
VEGF expression in retinal endothelial cells and Müller
cells exposed to hypoxia or high glucose and in retinas
from OIR and diabetic animals [12, 15]. Furthermore, we
showed that activation of ATF4 is required for hypoxia-
and ER stress-induced VEGF expression [15]. More recently,
a study from Ghosh and associates revealed that all three
UPR branches, IRE1α-XBP1, PERK-ATF4, and ATF6, are
involved in VEGF-A induction, which indicates an important
role of the UPR in angiogenesis [58]. In contrast, Liu et al.
showed that hyperglycemia or VEGF activates the IRE1α
and ATF6 pathways but not the PERK pathway, promoting
VEGF expression in retinal endothelial cells [59]. Nakamura
and coworkers demonstrated that mild ER stress, induced by low-
dosage tunicamycin or thapsigargin, triggers proliferation
and migration of retinal endothelial cells and promotes reti-
nal neoangiogenesis via induction of GRP78 [60]. Future
investigations are needed to decipher the exact mechanisms
by which the UPR regulates endothelial angiogenic response
and retinal angiogenesis.

3.4. The UPR and Retinal Pigment Epithelium (RPE) Dysfunc-
tion. While the majority of research on diabetes-related BRB
dysfunction underlying the pathogenesis of diabetic macular
dema focuses on alterations in endothelial tight junction
complex and vascular leakage in DR, very few studies have
looked into changes in the RPE during diabetes. Yet there is
evidence from new studies as well as from studies conducted
over 20 and 30 years ago suggesting that diabetes-associated
damage exists in the RPE, both in animal models and in
diabetic patients [61–63]. However, the effects of diabetes
on the RPE barrier and other important RPE functions
are poorly understood. In 2002, Abcouwer and colleagues
reported that amino acid deprivation induces ER stress,
resulting in VEGF upregulation in RPE cells [64]. This paper is one of the earliest studies that introduces ER stress and the UPR into retinal research and investigates a potential role of the UPR in VEGF regulation in RPE cells. In a later study, the same group demonstrated that activation of ATF4 is implicated in homocysteine-induced VEGF expression in RPE cells [65]. Furthermore, a recent study by Miranda and colleagues identified increased phosphorylation of eIF2α, which reflects activation of the PERK UPR branch, in RPE from diabetic patients with nonproliferative DR [66]. Similarly, Du and colleagues demonstrated that the ER chaperone GRP78 expression is increased in the RPE of diabetic subjects with retinopathy compared to those without retinopathy [17]. The role of ER stress and the UPR in diabetes-related RPE injury and potential implication in RPE-photoreceptor degeneration warrants future investigation.

3.5. The UPR and Retinal Neuronal Dysfunction. Eyes with DR exhibit neuronal dysfunction, including retinal ganglion cell (RGC) death, apoptosis of cells in the inner nuclear layer (INL), loss of synapses and dendrites, and related alterations in synaptic activities, as well as activation of microglial and glial cells (Müller cell and astrocytes). Growing evidence indicates that ER stress is associated with retinal neuronal cell death in several disease models [10, 67, 68]. In an experimental model of chronic glaucoma, ER stress has been shown to contribute to RGC death through activation of the PERK/p-eIF2α/CHOP pathway [69]. Shimazawa and colleagues reported increased ER stress in the retina correlated with apoptosis induced by N-methyl-D-aspartate (NMDA) or by raising intraocular pressure (IOP) [70]. A recent study by Oshitari and colleagues shows that enhanced PERK and CHOP levels in retinal neurons correlate with apoptosis of RGCs in retinas from diabetic rats, while similar observations were made in non-diabetic retinas after exposure to high glucose [71, 72]. Apart from a role of ER stress and the UPR in RGCs, Wu and associates demonstrated that oxidized and glycated LDL induce apoptosis of retinal Müller cells by upregulating proteins involved in UPR signaling, such as p-eIF2α, GRP78, CHOP, and ATF6, while activating proapoptotic Bax, caspase-3, and PARP [73]. Recently, a study by Devi and colleagues shows that exposing Müller cells to chronic hyperglycemia triggers prolonged ER stress and activates autophagy [74]. These results are consistent with our observation of increased ATF4 and CHOP activation in Müller cells, which partially colocalize with activated Müller cells in diabetic retina, suggesting that ER stress and the UPR may play a role in regulation of cellular activity of Müller cells [15].

4. XBP1 and Diabetic Retinopathy

XBP1 was originally identified as a protein binding to the X-box motif in human major histocompatibility complex class II gene in early 1990s [75]. A decade later, its regulating effects on the UPR were demonstrated by two laboratories independently, as the mammalian homolog of HAC1 in yeast, which was spliced by IRE1α [24, 27, 76]. Over the last 10 years, the role of XBP1 splicing/activation in cell fate determination during stress has been studied extensively. XBP1 is a basic-region leucine zipper protein in the cAMP responsive element binding protein/activating transcription factor (CREB/ATF) family of transcription factors and regulates a subset of UPR target genes [30]. It is expressed ubiquitously in adult tissues as well as in fetal exocrine glands and osteoblasts [77]. XBP1 is essential for hepatocyte growth, as global knockout of XBP1 embryos die in day 12.5 from severe liver hypoplasia and fetal anemia [78]. It is also required for ER expansion and the development of highly secretory cells such as plasma cells and pancreatic and salivary gland epithelial cells [79]. XBP1-/- mice exhibit increased ER stress coupled with impaired glucose and insulin tolerance during high-fat diet (HFD)-induced obesity [7, 8]. Accumulating evidence also implicates XBP1 splicing in many disease processes such as cancer, neurodegenerative diseases, and diabetes [8, 80, 81]. Novel roles of the IRE1α-XBP1 branch in inflammation, cell survival, apoptosis, and angiogenesis have been revealed in recent years [21, 51, 82], and more are likely to be discovered. In diabetic Akita mice, ER stress was increased in the retina [12] accompanied by increased XBP1 splicing (Figure 2). Furthermore, preconditioning with ER stress activating XBP1 protects retinal endothelial cells from TNF-α-induced inflammation and retinal vascular leakage and leukostasis [51]. In the following section, we will discuss the role of XBP1 in retinal vascular and neuronal cells and its potential role in DR development as well as in future treatment strategies.

4.1. XBP1 and Inflammation. Accumulating evidence shows that XBP1 splicing is implicated in inflammation. Mice that lack XBP1 in intestinal epithelial cells develop spontaneous inflammation in small intestine and exhibit pathological features of human inflammatory disease (IBD). These changes are accompanied by increased ER stress and enhanced susceptibility to inflammatory stimuli [83]. In the same study, an association between XBP1 variants and both forms of human IBD was identified [83]. Mechanistically, XBP1 deficiency causes increased ER stress and IRE1α activation, which recruits TRAF2 and induces activation of JNK and NF-κB [83]. These findings are consistent with an earlier study that demonstrates increased ER stress and JNK activation in the liver and adipose tissues, coupled with insulin resistance, in heterozygous XBP1 knockout mice challenged with a high-fat diet [8]. Likewise, heterozygous XBP1 knockout mice are more susceptible to alcohol-induced pancreatitis due to increased acinar cell apoptosis and inflammation [84]. In retinal endothelial cells, activation of XBP1 by preconditioning with ER stress attenuates TNF-α-induced inflammation and adhesion molecule expression [51]. Similarly, overexpressing spliced XBP1 sufficiently reduces TNF-α-induced IRE1α/IKK/NF-κB activation and leukocyte-endothelial cell adhesion [51]. In these cell types, XBP1 appears to be an important modulator of inflammatory response and suppresses overt inflammation in the retina and other tissues. However, in other studies, activation of the IRE1/XBP1 UPR by mild ER stress predisposes rat pancreatic β-cells to an exacerbated inflammatory reaction to IL-1β (but not TNF-α) through regulation of the transcription factor fork head box O-1 (FOXO-1) and NF-κB activity [85]. Activation of
XBPI also regulates production of microRNAs which in turn regulate target genes involved with immune response, such as the ER antigen peptide transporter 1 (TAP1), a key protein that coordinates the MHC class I-associated antigen presentation [86]. Discrepancies in the findings regarding the role of XBPI in inflammation and immune response may reflect the complex and multifaceted function of XBPI in regulation of the transcriptome.

4.2. XBPI and Angiogenesis. Several studies have reported a potential role of XBPI in the regulation of angiogenesis, yet the mechanism by which XBPI regulates endothelial cell activity and the angiogenic process remains poorly understood. In a recent study, Liu and colleagues demonstrated that targeting the IRE1α or ATF6 arms of the UPR by siRNA knockdown of IRE1α or ATF6 significantly reduces VEGF-stimulated angiogenic response of endothelial cells and diminishes angiogenesis in mouse models of OIR or choroidal neovascularization [59]. These results are exciting in that activation of the UPR by ER stress in endothelial cells challenged with multiple potent stimulants of angiogenesis such as VEGF, hypoxia and hydrogen peroxide is clearly important for retinal angiogenesis. However, activated IRE1α demonstrates kinase activity that recruits and activates stress kinases such as JNK and IKK. These kinases play a proangiogenic role, independent of XBPI splicing. Thus, future studies that separate kinase activity from endoribonuclease activity of IRE1α are needed to further refine the downstream pathways of IRE1 in mediating its pro-angiogenic and pro-VEGF effects.

In order to examine the role of XBPI in endothelial cells, Zeng and associates generated endothelial cell-specific XBPI knockout (XBPIecko) mice. These mice demonstrated impaired angiogenesis in response to ischemia [87]. Furthermore, XBPIs regulated endothelial cell proliferation in a PI3K/Akt/GSK3β/β-catenin/E2F2-dependent manner [87]. In contrast, Nakamura and colleagues showed that low doses of ER stress inducers, such as tunicamycin or thapsigargin, increase the proliferation and migration of retinal endothelial cells and induce retinal neovascularization without activation of XBPI splicing [60]. Thus, the exact role of XBPI in angiogenesis and retinal NV is yet to be confirmed.

4.3. XBPI and Autophagy. During ER stress, ERAD is activated to eliminate unfolded and misfolded protein aggregates. While misfolded polypeptides are transported to proteasome (ERAD I), large protein aggregates are primarily processed through the ER, activating autophagy (ERAD II). A low level of constitutive autophagy is also important for maintaining the quality of proteins and organelles, in order to maintain cellular function. Autophagy also serves as a crucial element of the stress response needed to protect β-cells under insulin-resistant states, and impaired autophagic machinery predisposes individuals to type 2 diabetes [88]. XBPIs activate transcription of major genes encoding ERAD components, such as ERdj4 [89], HRD1 [90], EDEM (ER degradation enhancing α-mannosidase like protein) and EDEM2 [91]. Fouillet et al. found that autophagy induced by mild ER stress selectively activates XBPI splicing, which provides neuroprotection in Drosophila and a mouse model of Parkinson disease [92]. Interestingly, XBPI activation in endothelial cells triggers autophagy, while knockdown of XBPI abolishes autophagic response at the basal level or in the presence of endostatin-1 [93]. However, uncontrolled autophagy can be detrimental and cause autophagy cell death (type 2 programmed cell death). Hetz and colleagues demonstrated that
downregulation of XBP1 results in uncontrolled autophagy [94, 95]. Induction of autophagy/ERAD II by the IRE1α/XBP1 pathway likely involves activating the ER membrane transporter AT-1 and acetylation of the autophagy protein Atg9A [94]. Thus, it appears that the IRE1α/XBP1 pathway plays a key role in integrating the UPR, ERAD, and autophagy to regulate cellular stress response. This notion is also supported by recent work by Adolph and colleagues demonstrating that deletion of XBP1 in intestinal epithelial cells results in ER stress, which activates PERK/eIF2α/ATF4 pathway and augments autophagy and somatostatin expression in duodenal homebox 1 (PDX1) and musculoaponeurotic fibrosarcoma oncogene homologue A (MAFA) [82].

Other gene products involved in XBP1 regulation during ER stress-induced apoptosis are the Bcl-2 family of proteins and XBP1 SUMOylation [104, 105]. SUMO is a novel ubiquitin-like protein that can covalently modify a large number of proteins and is reversed by a family of Sentrin/SUMO-specific proteases (SENPs). XBP1 can be SUMOylated during ER stress, which represses the transcriptional activity of XBP1 and leads to cell apoptosis. In recent studies, it was demonstrated that SENPI can increase the transcriptional activity of XBP1 through regulating XBP1 SUMOylation. Loss of SENPI enhances XBP1 SUMOylation resulting in ER stress and apoptosis [104]. Rodriguez and colleagues found that cells lacking BIM and PUMA, two novel members of Bcl-2 family of proteins, failed to maintain sustained XBP-1 splicing after prolonged ER stress that caused early inactivation of XBP1 [105]. Mutation in the BH3 domain of BIM abrogates its physical interaction with IRE1α, resulting in inhibition of XBP-1 splicing and reduced expression of XBP1-target genes [105]. With a Drosophila model for autosomal dominant retinitis pigmentosa, Ryoo and colleagues examined the role of XBP1 in photoreceptor cells. Their results show that reducing the expression of XBP1 accelerates photoreceptor loss and retinal degeneration [20]. In hippocampi from db/db mice, the expression of XBP1s was downregulated at both mRNA and protein levels after 24 weeks of diabetes but not in early diabetes (8 weeks), implying a potential role of XBP1 dysfunction in long-term diabetes-induced cognitive decline [81]. In contrast, overexpression of XBP1 protects RPE cells [21] and RGCs [106] from apoptosis induced by cytotoxin or axonal injury. These studies revealed the protective role of XBP1 in retinal neurons and the central nervous system. Enhancing XBP1 activity may offer a novel therapeutic strategy against retinal neuronal degeneration.

4.4. XBP1 and Retinal Cell Survival. ER stress orchestrates the process of cell death and survival depending upon the extent of UPR activation coupled with specific pathway components. It has been shown that activated XBP1 targets genes such as ER chaperones and genes responsible for ER-associated degradation (ERAD), such as P58IPK and PDI-5, to aid cell survival during UPR [100]. A recent study from Ryoo and colleagues demonstrates that deletion of XBP1 in Drosophila augments photoreceptor degeneration in ninaE G69D−/+, a Drosophila model for autosomal dominant retinitis pigmentosa (ADRP) suggesting that XBP1 is essential for photoreceptor survival during ER stress [20]. Our investigation demonstrated that XBP1s protects RPE and photoreceptor survival from light-induced apoptosis by suppressing oxidative stress and ER stress [21]. Consistently, mild ER stress (ER stress preconditioning) induces a selective activation of XBP1, which exerts a protective effect for cell survival [101, 102]. By contrast, sustained and full-fledged UPR involving GADD153/CHOP and ASPI2/caspase 12 activation is detrimental to the cell [10]. In addition, the level and duration of XBP1 activation appears to be important for downstream signaling events and biological effects on cell fate. For example, Zeng and colleagues reported that transient activation of XBP1 splicing may increase EC proliferation, while sustained activation leads to EC apoptosis, endothelium denudation, and atherosclerotic lesion development via multiple caspase activation, as well as downregulation of VE-cadherin through gene transcriptional effects and MMP-mediated degradation [103]. Sustained expression of XBP1s induces β-cell dysfunction and apoptosis via inhibition of insulin, duodenal homeobox 1 (PDX1) and musculoaponeurotic fibrosarcoma oncogene homologue A (MAFA) [82].

4.5. XBP1 and Oxidative Stress. The ER possesses a unique oxidizing environment that favors disulfide bond formation during the process of protein folding. Generation of reactive oxygen species (ROS) is a byproduct of this process. ER stress-initiated ROS generation amplifies mitochondrial ROS production and stimulates oxidative stress, which in turn enhances the ER stress response, resulting in a vicious cycle of cellular damage. Sustained ER stress and oxidative stress can lead to cell dysfunction or even cell death. Oxidative stress plays a pivotal role in the development of DR and also contributes to the resistance in reversing retinopathy after reinitiation of good glycemic control, a phenomenon known as metabolic memory [107]. Recent studies suggest UPR signaling regulates oxidative stress through transcriptional regulation of antioxidant genes. In mouse embryonic fibroblasts, deletion of XBP1 resulted in decreased expression of several antioxidant genes, including catalase, SOD1, and TRX1, augmented ROS generation and maintained P38 phosphorylation [108]. Overexpression of XBP1 restored catalase levels in XBP1-deficient cells and diminished ROS generation. Mutation analysis of the catalase promoter region revealed...
a potential site for direct regulation by XBP1 [108]. In RPE-specific XBP1 KO mice, the expression of antioxidant genes (SOD1, SOD2, and catalase) was significantly decreased; in contrast, the ROS level was markedly increased accompanied by enhanced oxidative damage and apoptosis of RPE cells. However, overexpression of spliced XBP1 failed to increase the expression of these antioxidant genes in RPE cells, suggesting the possibility that XBP1 indirectly regulates these genes in RPE [21]. Shao and associates reported that high glucose treatment significantly reduced XBP protein in renal mesangial cells, and simultaneously increased expression of the cytosolic NADPH oxidase subunit p47phox and ROS generation [109]. Overexpression of XBP1 reversed HG-induced ROS production and reduced p47phox expression, while knockdown of XBP1 led to increased ROS [109]. These findings suggest a potential role for XBP1 in maintaining cellular redox homeostasis by regulation of ROS-generating enzymes such as NADPH oxidase and antioxidant molecules; however, the exact mechanism is still not clear.

4.6. XBP1 and Glial Activation. Recent studies show that XBP1 splicing is involved in regulation of innate immunity [110]. Toll like receptors (TLRs) are part of the innate immune system that recognizes pathogen-associated molecules, induces inflammatory responses essential for host defenses, and initiates an adaptive immune response. TLRs are expressed in retinal cells, including RPE, microglia, Müller cells, and astrocytes [111, 112], while their role in glial activation has been studied in uveitis and age-related macular degeneration [113–115]. Retinal glial cells (Müller cells and astrocytes) and microglia are considered resident innate immune cells in the retina. These cells produce proinflammation cytokines, such as TNF-α, IL-1β, IL-8, IL-6 and ICAM in pathological conditions through activation of pathways including TLRs and a MyD88-dependent signaling cascade [113, 116]. Intravitreal injection of TLR2 agonist or Staphylococcus aureus activated TLR2 in Müller cells, resulting in the activation of NF-κB and P38 MAPK in mouse retina [117]. Activation of glial cells and microglia also contributes to the pathological progression of DR, including retinal inflammation, BRB breakdown and neuronal dysfunction [118–120]. Recent studies suggest that high glucose increases oxidative stress through downregulation of thioredoxin interacting/inhibiting protein (TXNIP) in Müller cells [74]. TXNIP, also known as vitamin D3 upregulated protein (VDUPI), was first identified as an endogenous inhibitor of thioredoxin, an ROS scavenger that maintains protein cysteine sulfhydryl groups by its redox-active disulfide/dithiol sites [74]. Exposing rat Müller cells to high glucose for five days resulted in sustained upregulation of TXNIP accompanied by increased ROS generation, ATP depletion, ER stress, autophagy, inflammation and apoptosis [74]. These results indicate that a complex network of interdependent intracellular signaling pathways may contribute to diabetes-induced alternations in glial cells. In our recent study, we demonstrated that activation of ER stress by high glucose leads to increased VEGF and adhesion molecule expression through induction of ATF4 in Müller cells [15]. How other UPR branches (i.e., IRE/XBP1 and ATF6) are implicated in Müller cell activation and inflammation is currently unknown. Given the anti-inflammatory action of XBP1 in retinal endothelial cells [51], we speculate that activation of XBP1 during ER stress could reduce inflammatory cytokine production and attenuate glial cell activation in DR. Intriguingly, a recent study shows that XBP1 activation by ER stress in macrophages enhances TLR signaling and innate immune response [121]. It would be of great interest to elucidate how XBP1 regulate retinal glial cell activation and related inflammation in DR.

5. Conclusions and Perspectives

Although many clinical trials on novel therapeutic strategies have been conducted or are currently ongoing, DR remains the leading cause of blindness in working-age adults in the US. Better understanding of the cellular and molecular mechanisms underlying diabetes-related vascular and neuronal damage is important to identify therapeutic targets to protect the retina and reduce vision loss in diabetic patients. Emerging evidence suggests that increased ER stress and activation of the UPR in retinal cells promote the main pathophysiological events in DR, such as oxidative stress, inflammation, apoptosis, vascular leakage, and angiogenesis (Figure 3). Among the UPR pathways, the IRE1α/XBP1 branch is considered to be the core check point, and a key determinant of cell fate. Despite many elegant studies over the past decade demonstrating a crucial role of XBP1s in...
regulating inflammation, angiogenesis, and cell death, the exact functions of XBP1 in specific types of retinal cells and its implication in retinal adaptive response and retinal cell injury during the course of DR development and progression remain poorly understood. In addition, there is clear evidence of active cross-talk among signaling pathways of the UPR and between UPR signaling and other cellular stress responses. These complex and dynamic networks tightly control cell homeostasis and contribute to the pathogenesis of many diseases. Therefore, investigation of XBP1 in a broader range of active cross-talk among signaling pathways of the UPR and remain poorly understood. In addition, there is clear evidence of exact functions of XBP1 in specific types of retinal cells and regulating inflammation, angiogenesis, and cell death, the exact functions of XBP1 in specific types of retinal cells and its implication in retinal adaptive response and retinal cell injury during the course of DR development and progression remain poorly understood. In addition, there is clear evidence of active cross-talk among signaling pathways of the UPR and between UPR signaling and other cellular stress responses. These complex and dynamic networks tightly control cell homeostasis and contribute to the pathogenesis of many diseases. Therefore, investigation of XBP1 in a broader range of active cross-talk among signaling pathways of the UPR and remain poorly understood. In addition, there is clear evidence of exact functions of XBP1 in specific types of retinal cells and regulating inflammation, angiogenesis, and cell death, the exact functions of XBP1 in specific types of retinal cells and its implication in retinal adaptive response and retinal cell injury during the course of DR development and progression remain poorly understood. In addition, there is clear evidence of active cross-talk among signaling pathways of the UPR and between UPR signaling and other cellular stress responses. These complex and dynamic networks tightly control cell homeostasis and contribute to the pathogenesis of many diseases. Therefore, investigation of XBP1 in a broader range of cellular events may help us understand its role in the retina and provide new insights into the mechanisms of retinal pathology in DR.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**

[1] D. R. Whiting, L. Guariguata, C. Weil, and J. Shaw, “IDF Diabetes Atlas: global estimates of the prevalence of diabetes for 2011 and 2030,” *Diabetes Research and Clinical Practice*, vol. 94, no. 3, pp. 311–321, 2011.

[2] M. Rema, S. Premkumar, B. Anitha, R. Deepa, R. Pradeepa, and V. Mohan, “Prevalence of diabetic retinopathy in urban India: the Chennai urban rural epidemiology study (CURES) eye study, I: Investigative Ophthalmology & Visual Science*, vol. 46, no. 7, pp. 2328–2333, 2005.

[3] J. H. Kempen, B. J. O’Colmain, M. C. Leske et al., “The prevalence of diabetic retinopathy among adults in the United States,” *Archives of Ophthalmology*, vol. 122, no. 4, pp. 552–563, 2004.

[4] N. Congdon, B. O’Colmain, C. C. Klaver et al., “Causes and prevalence of visual impairment among adults in the United States,” *Archives of Ophthalmology*, vol. 122, no. 4, pp. 477–485, 2004.

[5] F. L. Ferris III, “Results of 20 years of research on the treatment of diabetic retinopathy,” *Preventive Medicine*, vol. 23, no. 5, pp. 740–742, 1994.

[6] D. M. Nathan, B. Zinman, P. A. Cleary et al., “Modern-day clinical course of type 1 diabetes mellitus after 30 years’ duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983–2005),” *Archives of Internal Medicine*, vol. 169, no. 14, pp. 1307–1316, 2009.

[7] U. Ozcan, E. Yilmaz, L. Ozcan, and et al., “Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes,” *Science*, vol. 313, no. 5790, pp. 1137–1140, 2006.
S. J. Marciniak, C. Y. Yun, S. Oyadomari et al., “CHOP induces M. M. U. Ali, T. Bagratuni, E. L. Davenport et al., “Structure of A.-H. Lee, N. N. Iwakoshi, and L. H. Glimcher, “XBP-1 regulates M. Kanemoto, S. Kondo, M. Ogata, T. Murakami, F. Urano, K. Haze, H. Yoshida, H. Yanagi, T. Yura, and K. Mori, “A-time-dependent phase shift in the mammalian unfolded protein response,” Developmental Cell, vol. 4, no. 2, pp. 265–271, 2003.

S. Kanemoto, S. Kondo, M. Ogata, T. Murakami, F. Urano, and K. Imaiizumi, “XBP1 activates the transcription of its target genes via an ACGT core sequence under ER stress,” Biochemical and Biophysical Research Communications, vol. 331, no. 4, pp. 1146–1153, 2005.

A.-H. Lee, N. N. Iwakoshi, and L. H. Glimcher, “XBP-1 regulates a subset of an endoplasmic reticulum resident chaperone genes in the unfolded protein response,” Molecular and Cellular Biology, vol. 23, no. 21, pp. 7448–7459, 2003.

H. P. Harding, Y. Zhang, and D. Ron, “Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase,” Nature, vol. 397, pp. 271–274, 1999.

H. P. Harding, Y. Zhang, H. Zeng et al., “An integrated stress response regulates amino acid metabolism and resistance to oxidative stress,” Molecular Cell, vol. 11, no. 3, pp. 619–633, 2003.

J. L. Steiger, S. Bandyopadhyay, D. H. Farb, and S. J. Russek, cAMP response element–binding protein, activating transcription factor–4, and upstream stimulatory factor differentially control hippocampal GABA<sub>A</sub>Ria and GABA<sub>B</sub>Rib subunit gene expression through alternative promoters,” Journal of Neuroscience, vol. 24, no. 27, pp. 615S–6162S, 2004.

S. J. Marciniak, C. Y. Yun, S. Oyadomari et al., “CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum,” Genes & Development, vol. 18, no. 24, pp. 3066–3077, 2004.

P. Tsaytler, H. P. Harding, D. Ron, and A. Bertolotti, “Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis,” Science, vol. 332, no. 6025, pp. 91–94, 2011.

B. F. Teske, S. A. Wek, P. Bunpo et al., “The eIF2 kinase PERK and the integrated stress response facilitate activation of ATF6 during endoplasmic reticulum stress,” Molecular Biology of the Cell, vol. 22, no. 22, pp. 4390–4405, 2011.

A. J. Schindler and R. Schekman, “In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 42, pp. 17775–17780, 2009.

K. Haze, H. Yoshida, H. Yanagi, T. Yura, and K. Mori, “Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress,” Molecular Biology of the Cell, vol. 10, no. 11, pp. 3787–3799, 1999.

J. Y. Ye, R. B. Rawson, R. Komuro et al., “ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs,” Molecular Cell, vol. 6, no. 6, pp. 1355–1364, 2000.

J. A. Vekich, P. J. Belmont, D. J. Thuerauf, and C. C. Glombotski, “Protein disulfide isomerase-associated 6 is an ATF6-inducible ER stress response protein that protects cardiac myocytes from ischemia/reperfusion-mediated cell death,” Journal of Molecular and Cellular Cardiology, vol. 53, no. 2, pp. 259–267, 2012.

Y. Wang, J. Shen, N. Arenzana, W. Tirasophon, R. J. Kaufman, and R. Prywes, “Activation of ATF6 and an ATF6 DNA binding site by the endoplasmic reticulum stress response,” The Journal of Biological Chemistry, vol. 275, no. 35, pp. 27013–27020, 2000.

H. Yoshida, T. Okada, K. Haze et al., “ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response,” Molecular and Cellular Biology, vol. 20, no. 18, pp. 6755–6767, 2000.

J. Wu, D. T. Rutkowski, M. Dubois et al., “ATF6α optimizes long-term endoplasmic reticulum function to protect cells from chronic stress,” Developmental Cell, vol. 13, no. 3, pp. 351–364, 2007.

K. Yamamoto, T. Sato, T. Matsui et al., “Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1,” Developmental Cell, vol. 13, no. 3, pp. 365–376, 2007.

D. A. Antonetti, R. Klein, and T. W. Gardner, “Diabetic retinopathy,” The New England Journal of Medicine, vol. 366, no. 13, pp. 1227–1239, 2012.

D. A. Antonetti, E. Lieth, A. J. Barber, and T. W. Gardner, “Molecular mechanisms of vascular permeability in diabetic retinopathy,” Seminars in Ophthalmology, vol. 14, no. 4, pp. 240–248, 1999.

C. J. Pournaras, E. Rungger-Brändle, C. E. Riva, S. H. Hardeson, and E. Stefansson, “Regulation of retinal blood flow in health and disease,” Progress in Retinal and Eye Research, vol. 27, no. 3, pp. 284–330, 2008.

S. X. Zhang and J.-X. Ma, “Ocular neovascularization: implication of endogenous angiogenic inhibitors and potential therapy,” Progress in Retinal and Eye Research, vol. 26, no. 1, pp. 1–37, 2007.

T. Yoshimura, K.-H. Sonoda, M. Sugahara et al., “Comprehensive analysis of inflammatory immune mediators in vitreoretinal diseases,” PLoS ONE, vol. 4, no. 12, Article ID e8158, 2009.

E. A. Pierce, R. L. Avery, E. D. Foley, L. P. Aiello, and L. E. H. Smith, “Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization,” Proceedings of the National Academy of Sciences of the United States of America, vol. 92, no. 3, pp. 905–909, 1995.

J. Li, J. J. Wang, and S. X. Zhang, “Preconditioning with endoplasmic reticulum stress mitigates retinal endothelial inflammation via activation of X-box binding protein 1,” The Journal of Biological Chemistry, vol. 286, no. 6, pp. 4912–4921, 2011.

K. Ikeshig, M. L. Mulhern, C. J. Madson et al., “Induction of endoplasmic reticulum stress in retinal pericytes by glucose deprivation,” Current Eye Research, vol. 31, no. 11, pp. 947–953, 2006.

Y. Zhong, J. J. Wang, and S. X. Zhang, “Intermittent but not constant high glucose induces ER stress and inflammation in human retinal pericytes,” Advances in Experimental Medicine and Biology, vol. 723, pp. 285–292, 2012.

T. Adachi, H. Yasuda, K. Aida et al., “Regulation of extracellular superoxide dismutase in rat retina pericytes,” Redox Report, vol. 15, no. 6, pp. 250–258, 2010.
[55] F. G. Holz, S. Schmitz-Valckenberg, and M. Fleckenstein, “Recent developments in the treatment of age-related macular degeneration,” The Journal of Clinical Investigation, vol. 124, no. 4, pp. 1430–1438, 2014.

[56] D. Dong, M. Ni, J. Li et al., “Critical role of the stress chaperone GRP78/Bip in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development,” Cancer Research, vol. 68, no. 2, pp. 498–505, 2008.

[57] B. Li, D. Li, G.-G. Li, H.-W. Wang, and A.-X. Yu, “PS8K inhibition of endoplasmic reticulum stress in human retinal capillary endothelial cells in vitro,” Molecular Vision, vol. 14, pp. 1122–1128, 2008.

[58] R. Ghosh, K. L. Lipson, K. E. Sargent et al., “Transcriptional regulation of VEGF-A by the unfolded protein response pathway,” PLoS ONE, vol. 5, no. 3, Article ID e9575, 2010.

[59] L. Liu, X. Qi, Z. Chen et al., “Targeting the IRE1α/XBPI and ATF6 arms of the unfolded protein response enhances VEGF blockade to prevent retinal and choroidal neovascularization,” The American Journal of Pathology, vol. 182, no. 4, pp. 1412–1424, 2013.

[60] S. Nakamura, H. Takizawa, M. Shimazawa et al., “Mild endoplasmic reticulum stress promotes retinal neovascularization via induction of Bip/GRP78,” PLoS ONE, vol. 8, no. 3, Article ID e60517, 2013.

[61] W. M. Kirber, C. W. Nichols, P. A. Grimes, A. I. Winegrad, and A. M. Laties, “A permeability defect of the retinal epithelium. Occurrence in early streptozotocin diabetes,” Archives of Ophthalmology, vol. 98, no. 4, pp. 725–728, 1980.

[62] S. A. Vinores, C. Gadegbeku, P. A. Campochiaro, and W. R. Green, “Immunohistochemical localization of blood-retinal barrier breakdown in human diabetics,” The American Journal of Pathology, vol. 134, no. 2, pp. 231–235, 1989.

[63] H.-Z. Xu and Y.-Z. Le, “Significance of outer blood-retina barrier breakdown in diabetes and ischemia,” Investigative Ophthalmology & Visual Science, vol. 52, no. 5, pp. 2160–2164, 2011.

[64] S. F. Abcouver, P. L. Marjon, R. K. Loper, and D. L. Vander Jagt, “Response of VEGF expression to amino acid deprivation and inducers of endoplasmic reticulum stress,” Investigative Ophthalmology and Visual Science, vol. 43, no. 8, pp. 2791–2798, 2002.

[65] C. N. Roybal, S. Yang, C.-W. Sun et al., “Homocysteine increases the expression of vascular endothelial growth factor by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4,” The Journal of Biological Chemistry, vol. 279, no. 15, pp. 14844–14852, 2004.

[66] S. Miranda, Á. González-Rodríguez, M. García-Ramírez et al., “Beneficial effects of fenofibrate in retinal pigment epithelium by the modulation of stress and survival signaling under diabetic conditions,” Journal of Cellular Physiology, vol. 227, no. 6, pp. 2352–2362, 2012.

[67] H. Liu, J. Qian, F. Wang et al., “Expression of two endoplasmic reticulum stress markers, GRP78 and GADD153, in rat retinal detachment model and its implication,” Eye, vol. 24, no. 1, pp. 137–144, 2010.

[68] N. Hata, T. Oshitarı, A. Yokoyama, Y. Mitamura, and S. Yamamoto, “Increased expression of IRE1α and stress-related signal transduction proteins in ischemia-reperfusion injured retina,” Clinical Ophthalmology, vol. 2, pp. 743–752, 2008.

[69] S. H. Doh, J. H. Kim, K. M. Lee, H. Y. Park, and C. K. Park, “Retinal ganglion cell death induced by endoplasmic reticulum stress in a chronic glaucoma model,” Brain Research, vol. 1308, pp. 158–166, 2010.

[70] M. Shimazawa, Y. Inokuchi, Y. Ito et al., “Involvement of ER stress in retinal cell death,” Molecular Vision, vol. 13, pp. 578–587, 2007.

[71] T. Oshitari, N. Yoshida-Hata, and S. Yamamoto, “Effect of neurotrophin-4 on endoplasmic reticulum stress-related neuronal apoptosis in diabetic and high glucose exposed rat retinas,” Neuroscience Letters, vol. 501, no. 2, pp. 102–106, 2011.

[72] T. Oshitari, N. Hata, and S. Yamamoto, “Endoplasmic reticulum stress and diabetic retinopathy,” Vascular Health and Risk Management, vol. 4, no. 1, pp. 115–122, 2008.

[73] M. Wu, S. Yang, M. H. Elliott et al., “Oxidative and endoplasmic reticulum stress mediates apoptosis induced by modified LDL in human retinal Müller cells,” Investigative Ophthalmology and Visual Science, vol. 53, no. 8, pp. 4595–4604, 2012.

[74] T. S. Devi, I. Lee, M. Hüttemann, A. Kumar, K. D. Nantwi, and L. P. Singh, “TXNIP links innate host defense mechanisms to oxidative stress and inflammation in retinal muller glia under chronic hyperglycemia: implications for diabetic retinopathy,” Experimental Diabetes Research, vol. 2012, Article ID 438238, 19 pages, 2012.

[75] H.-C. Liou, M. R. Boothby, P. W. Finn et al., “A new member of the leucine zipper class of proteins that binds to the HLA DRα promoter,” Science, vol. 247, no. 4950, pp. 1581–1584, 1990.

[76] X. Shen, R. E. Ellis, K. Lee et al., “Complementary signaling pathways regulate the unfolded protein response and are required for C. elegans development,” Cell, vol. 107, no. 7, pp. 893–903, 2001.

[77] I. M. Clauss, E. M. Gravallese, J. M. Darling, F. Shapiro, M. J. Glimcher, and L. H. Glimcher, “In situ hybridization studies suggest a role for the basic region-leucine zipper protein hXBP-1 in exocrine gland and skeletal development during mouse embryogenesis,” Developmental Dynamics, vol. 197, no. 2, pp. 146–156, 1993.

[78] A. M. Reimold, A. Etkin, I. Clauss et al., “An essential role in liver development for transcription factor XBP-1,” Genes and Development, vol. 14, no. 2, pp. 152–157, 2000.

[79] R. Sriburi, S. Jackowski, K. Mori, and J. W. Brewer, “XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum,” Journal of Cell Biology, vol. 167, no. 1, pp. 35–41, 2004.

[80] K. D. Tardif, K. Mori, R. J. Kaufman, and A. Siddiqui, “Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response,” Journal of Biological Chemistry, vol. 279, no. 17, pp. 17158–17164, 2004.

[81] C. Sims-Robinson, S. Zhao, J. Hur, and E. L. Feldman, “Central nervous system endoplasmic reticulum stress in a murine model of Type 2 diabetes,” Diabetologia, vol. 55, no. 8, pp. 2276–2284, 2012.

[82] F. Alagnat, F. Christulia, F. Ortis et al., “Sustained production of spliced X-box binding protein 1 (XBP1) induces pancreatic beta cell dysfunction and apoptosis,” Diabetologia, vol. 53, no. 6, pp. 1120–1130, 2010.

[83] A. Kaser, A.-H. Lee, A. Franke et al., “XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease,” Cell, vol. 134, no. 5, pp. 743–756, 2008.

[84] S. J. Pandol, F. S. Gorelick, A. Gerloff, and A. Lugea, “Alcohol abuse, endoplasmic reticulum stress and pancreatitis,” Digestive Diseases, vol. 28, no. 6, pp. 776–782, 2010.
Journal of Diabetes Research 13

[85] M. Miani, M. L. Colli, L. Ladrière, M. Cnop, and D. L. Eizirik, "Mild endoplasmic reticulum stress augments the proinflammatory effect of IL-1β in pancreatic rat β-cells via the IRE1α/XBP1S pathway," Endocrinology, vol. 153, no. 7, pp. 3017–3028, 2012.

[86] R. Bartoszewski, J. W. Brewer, A. Rab et al., "The Unfolded Protein Response (UPR)-activated transcription factor X-box binding protein 1 (XBPI) induces microRNA-346 expression that targets the human antigen peptide transporter 1 (TAP1) mRNA and governs immune regulatory genes," The Journal of Biological Chemistry, vol. 286, no. 48, pp. 41862–41870, 2011.

[87] L. Zeng, Q. Xiao, M. Chen et al., "Vascular endothelial cell growth-activated XBPI splicing in endothelial cells is crucial for angiogenesis," Circulation, vol. 127, no. 16, pp. 1712–1722, 2013.

[88] Y. Fujitani, R. Kawamori, and H. Watada, "The role of autophagy in pancreatic β-cell and diabetes," Autophagy, vol. 5, no. 2, pp. 280–282, 2009.

[89] C. W. Lai, J. H. Oteros, L. M. Hendershots, and E. Snapp, "ER stress inhibits neuronal death by promoting autophagy," Autophagy, vol. 8, no. 6, pp. 915–926, 2012.

[90] A. Margariti, H. Li, T. Chen et al., "XBPI mRNA splicing triggers an autophagic response in endothelial cells through BCLN1 transcriptional activation," The Journal of Biological Chemistry, vol. 288, no. 2, pp. 859–872, 2013.

[91] M. Pehar, M. C. Jonas, T. M. Hare, and L. Puglielli, "SLC33A1/AT-1 protein regulates the induction of autophagy downstream of IRE1α/XBP1 pathway," The Journal of Biological Chemistry, vol. 287, no. 35, pp. 29922–29930, 2012.

[92] S. Matus, M. Nassif, L. H. Glimcher, and C. Hetz, "XBPI deficiency in the nervous system reveals a homeostatic switch to activate autophagy," Autophagy, vol. 5, no. 8, pp. 1226–1228, 2009.

[93] T. E. Adolph, M. F. Tomczak, L. Niederreiter et al., "Paneth cells as a site of origin for intestinal inflammation," Nature, vol. 503, no. 7475, pp. 272–276, 2013.

[94] M. M. Kunte, S. Choudhury, J. F. Manheim et al., "ER stress is involved in T2M rhodopsin-induced retinal degeneration," Investigative Ophthalmology and Visual Science, vol. 53, no. 7, pp. 3792–3800, 2012.

[95] A. L. Wang, T. J. Lukas, M. Yuan, N. Du, M. O. Tso, and A. H. Neufeld, "Autophagy, exosomes and drusen formation in age-related macular degeneration," Autophagy, vol. 5, no. 4, pp. 563–564, 2009.

[96] K. Kaarniranta, "Autophagy—hot topic in AMD," Acta Ophthalmologica, vol. 88, no. 4, pp. 387–388, 2010.

[97] H. Yang, R. Liu, Z. Cui et al., "Functional characterization of 58-kilodalton inhibitor of protein kinase in protecting against diabetic retinopathy via the endoplasmic reticulum stress pathway," Molecular Vision, vol. 17, pp. 78–84, 2011.

[98] J. H. Lin, H. Li, D. Yasumura et al., "IRE1α signaling affects cell fate during the unfolded protein response," Science, vol. 318, no. 5882, pp. 944–949, 2007.

[99] C. S. Mendes, C. Levett, G. Chatelain et al., "ER stress protects from retinal degeneration," EMBO Journal, vol. 28, no. 9, pp. 1296–1307, 2009.

[100] L. Zeng, Q. Xiao, M. Chen et al., "Sustained activation of XBPI splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow," Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 20, pp. 8326–8331, 2009.

[101] Z. Jiang, Q. Fan, Z. Zhang et al., "SENPI deficiency promotes ER stress-induced apoptosis by increasing XBPI SUMOylation," Cell Cycle, vol. 11, no. 6, pp. 1118–1122, 2012.

[102] D. A. Rodriguez, S. Zamorano, F. Lisbona et al., "BH3-only proteins are part of a regulatory network that control the sustained signalling of the unfolded protein response sensor IRE1α," The EMBO Journal, vol. 31, no. 10, pp. 2322–2335, 2012.

[103] Y. Hu, K. Park, L. Yang et al., "Differential effects of unfolded protein response pathways on axon injury-induced death of retinal ganglion cells," Neuron, vol. 73, no. 3, pp. 445–452, 2012.

[104] R. A. Kowluru and P.-S. Chan, "Oxidative stress and diabetic retinopathy," Experimental Diabetes Research, vol. 2007, Article ID 43603, 12 pages, 2007.

[105] Y. Liu, M. Adachi, S. Zhao et al., "Preventing oxidative stress: a new role for XBPI," Cell Death & Differentiation, vol. 16, no. 6, pp. 847–857, 2009.

[106] D. Shao, J. Liu, J. Ni et al., "Suppression of XBPI mediates high glucose-induced oxidative stress and extracellular matrix synthesis in renal mesangial cell and kidney of diabetic rats," PLoS ONE, vol. 8, no. 2, Article ID e56124, 2013.

[107] C. E. Richardson, T. Kooistra, and D. H. Kim, "An essential role for XBPI-1 in host protection against immune activation in C. elegans," Nature, vol. 463, no. 7284, pp. 1092–1095, 2010.

[108] M. V. Kumar, C. N. Nagineni, M. S. Chin, J. J. Hooks, and B. Detrick, "Innate immunity in the retina: toll-like receptor (TLR) signaling in human retinal pigment epithelial cells," Journal of Neuroimmunology, vol. 153, no. 1–2, pp. 7–15, 2004.

[109] G. Jiang, Y. Ke, D. Sun, Y. Wang, H. J. Kaplan, and H. Shao, "Regulatory role of TLR ligands on the activation of autoreactive T cells by retinal astrocytes," Investigative Ophthalmology & Visual Science, vol. 50, no. 10, pp. 4769–4776, 2009.

[110] A. Kumar and N. Shamsuddin, "Retinal muller glia initiate innate response to infectious stimuli via toll-like receptor signaling," PLoS ONE, vol. 7, no. 1, Article ID e29830, 2012.

[111] Z. Yang, C. Stratton, P. J. Francis et al., "Toll-like receptor 3 and geographic atrophy in age-related macular degeneration," The New England Journal of Medicine, vol. 359, no. 14, pp. 1456–1463, 2008.

[112] G. Dvoriantchikova, D. J. Barakat, E. Hernandez, V. I. Shestopalov, and D. Ivanov, "Toll-like receptor 4 contributes to retinal ischemia/reperfusion injury," Molecular Vision, vol. 16, pp. 1907–1912, 2010.

[113] T. Kochan, A. Singla, J. Tosi, and A. Kumar, "Toll-like receptor 2 ligand pretreatment attenuates retinal microglial inflammatory response but enhances phagocytic activity toward Staphylococcus aureus," Infection and Immunity, vol. 80, no. 6, pp. 2076–2088, 2012.

[114] N. Shamsuddin and A. Kumar, "TLR2 mediates the innate response of retinal muller glia to Staphylococcus aureus," Journal of Immunology, vol. 186, no. 12, pp. 7089–7097, 2011.
[118] R. A. Feit-Leichman, R. Kinouchi, M. Takeda et al., "Vascular damage in a mouse model of diabetic retinopathy: relation to neuronal and glial changes," Investigative Ophthalmology and Visual Science, vol. 46, no. 11, pp. 4281–4287, 2005.

[119] H.-J. Ma, Y. Luo, M. Wang et al., "Changes of tight junction protein and GFAP in the retina of experimental diabetic rats and their relationship with blood aqueous barrier," Zhonghua Yan Ke Za Zhi, vol. 43, no. 5, pp. 397–401, 2007.

[120] V. H. Y. Wong, A. J. Vingrys, and B. V. Bui, “Gliarial and neuronal dysfunction in streptozotocin-induced diabetic rats,” Journal of Ocular Biology, Diseases, and Informatics, vol. 4, no. 1-2, pp. 42–50, 2011.

[121] F. Martinon, X. Chen, A.-H. Lee, and L. H. Glimcher, “TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages,” Nature Immunology, vol. 11, no. 5, pp. 411–418, 2010.

[122] C. Chen, M. Cano, J. J. Wang et al., “Role of unfolded protein response dysregulation in oxidative injury of retinal pigment epithelial cells,” Antioxidants and Redox Signaling, vol. 20, no. 14, pp. 2091–2106, 2014.

[123] C. Chen, J. J. Wang, J. Li, Q. Yu, and S. X. Zhang, “Quinotrikerixin inhibits proliferation of human retinal pigment epithelial cells,” Molecular Vision, vol. 19, pp. 39–46, 2013.

[124] H. Miyagi, S. Kanemoto, A. Saito et al., “Transcriptional regulation of VEGFA by the endoplasmic reticulum stress transducer OASIS in ARPE-19 cells,” PLoS ONE, vol. 8, no. 1, Article ID e55155, 2013.

[125] C. Nathaniel Roybal, L. A. Hunsaker, O. Barbash, D. L. Vander Jagt, and S. F. Abcouwer, "The oxidative stressor arsenite activates vascular endothelial growth factor mRNA transcription by an ATF4-dependent mechanism," Journal of Biological Chemistry, vol. 280, no. 21, pp. 20331–20339, 2005.

[126] M. Shimazawa, Y. Ito, Y. Inokuchi, and H. Hara, "Involvement of double-stranded RNA-dependent protein kinase in ER stress-induced retinal neuron damage," Investigative Ophthalmology & Visual Science, vol. 48, no. 8, pp. 3729–3736, 2007.