

# Endoplasmic reticulum stress and diabetic retinopathy

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**Abstract:** Endoplasmic reticulum (ER) stress is involved in the pathogenesis of several diseases including Alzheimer disease and Parkinson disease. Many recent studies have shown that ER stress is related to the pathogenesis of diabetes mellitus, and with the death of pancreatic  $\beta$ -cells, insulin resistance, and the death of the vascular cells in the retina. Diabetic retinopathy is a major complication of diabetes and results in death of both neural and vascular cells. Because the death of the neurons directly affects visual function, the precise mechanism causing the death of neurons in early diabetic retinopathy must be determined. The ideal therapy for preventing the onset and the progression of diabetic retinopathy would be to treat the factors involved with both the vascular and neuronal abnormalities in diabetic retinopathy. In this review, we present evidence that ER stress is involved in the death of both retinal neurons and vascular cells in diabetic eyes, and thus reducing or blocking ER stress may be a potential therapy for preventing the onset and the progression of diabetic retinopathy.

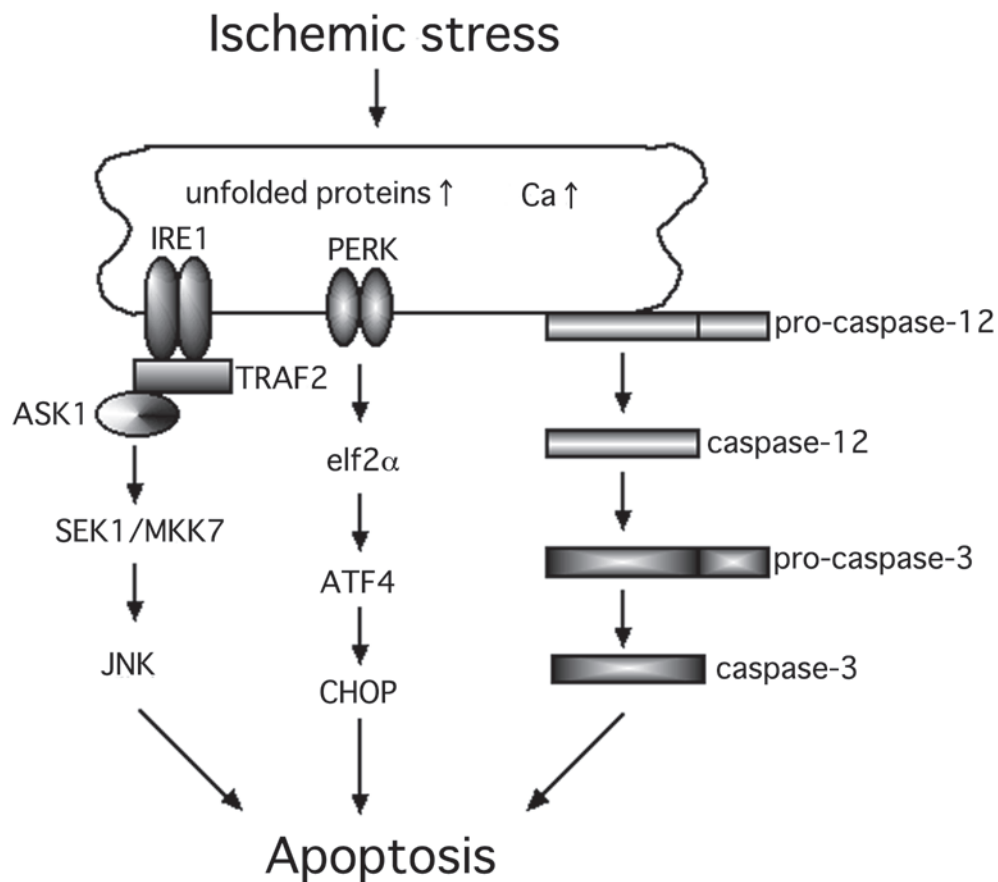
**Keywords:** endoplasmic reticulum stress, diabetic retinopathy, vascular cell death, neuronal cell death

## Introduction

Diabetic retinopathy is a major complication in patients with diabetes and it can lead to severe visual decrease in a high percentage of diabetic patients (Oshitari 2006; Oshitari and Roy 2007). Although the precise mechanism(s) for the onset and progression of diabetic retinopathy has still not been determined, recent studies have indicated that neuronal and vascular abnormalities are associated with the pathogenesis of early diabetic retinopathy (Barber et al 1998; Takano et al 1999; Asnaghi et al 2003; Martin et al 2004; Oshitari et al 2005). The neuronal abnormalities in the early stage of diabetic retinopathy are difficult to observe and evaluate by routine clinical tests, but ophthalmologists should consider neuronal abnormalities, including the death of retinal ganglion cell (RGCs), when evaluating eyes with diabetic retinopathy. This is important because the death of retinal neurons is irreversible and directly affects the visual function (Oshitari 2006; Oshitari and Roy 2007).

The endoplasmic reticulum (ER) is a critical intracellular organelle, which has several vital functions such as protein synthesis (Chevet et al 2001), protein transport (Palade 1975), and acts as a reservoir of  $\text{Ca}^{2+}$  (Nielsen and Podolsky 1972). The accumulation of unfolded proteins or an upset in the  $\text{Ca}^{2+}$  homeostasis in the ER will activate the ER stress response, eg, the unfolded protein response (UPR) and the ER overload response (Rao et al 2004; Lindholm et al 2006). Most importantly, ER stress activates several cell death pathways including the caspase-12-dependent pathway (Nakagawa et al 2000), apoptosis signal-regulating kinase 1 (ASK1) – c-Jun N-terminal kinase (JNK) pathway (Nishitoh et al 2002) and the PKR-like endoplasmic reticulum kinase (PERK) – C/ERB homologous protein (CHOP) pathway (Ma et al 2002) (Figure 1).

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**Figure 1** Hypothesized scheme of the ER stress-mediated cell death pathways. At least, three major cell death pathways are associated with ER stress-induced cell death. **Abbreviations:** ER, endoplasmic reticulum; TRAF2, tumor necrosis factor receptor-associated factor 2; ASK1, apoptosis signal-regulating kinase 1; SEK1, SAPK/ERK kinase 1; MKK7, mitogen-activated protein kinase kinase 7; JNK, c-Jun N-terminal kinase; ATF4, activating transcription factor 4; PERK, PKR-like endoplasmic reticulum kinase; elf2 $\alpha$ , a subunit of translation eukaryotic initiation factor 2; CHOP, C/ERB homologous protein.

The results of recent studies have shown that ER stress-mediated cell death is associated with the death of pancreatic  $\beta$ -cells in patients with diabetes (Oyadomari et al 2001, 2002; Laybutt et al 2007). In addition, a recent study reported that the ER stress-induced apoptosis is related to changes in the glucose concentration and results in the death of pericytes (Ikesugi et al 2006). Roybal and colleagues (2004) suggested that the ER stress-mediated activating transcriptional factor 4 (ATF4) activation was associated with an over-expression of vascular endothelial growth factor (VEGF). In addition, recent studies indicate that ER stress-induced apoptosis is involved in the death of neurons in the brain and retina under different physiological conditions (Guo et al 1997; Tobisawa et al 2003; Imai and Takahashi 2004; Larner et al 2004; Tajiri et al 2004; Wootz et al 2004; Hayashi et al 2005; Awai et al 2006; Shimazawa et al 2007).

One therapeutic strategy that might be used to prevent the development and progression of type 2 diabetes is the inhibition of ER stress. This would then block the ER

stress-induced pancreatic  $\beta$ -cell death, and may also prevent the onset and progression of diabetic retinopathy. We shall discuss the possible role of ER stress in the pathogenesis of diabetic retinopathy and describe potential therapeutic strategies to block the development and the progression of diabetic retinopathy.

## ER stress-mediated pancreatic $\beta$ -cell death in diabetes

The ER is involved in the maintenance of cellular homeostasis by inducing the UPR. The UPR of mammals is mediated by at least three types of ER transmembrane proteins; IRE1 (protein kinase and site-specific endoribonuclease) (Tirasophon et al 1998; Wang et al 1998), PERK (Harding et al 2000), and ATF6 (Ye et al 2000). Under diabetic conditions, the pancreatic  $\beta$ -cells are continuously exposed to oxidative stress, eg, exposure to reactive oxygen species (ROS; Kaneto et al 2005) and to nitrous oxide (NO; Oyadomari et al 2001). The ER is highly developed in pancreatic  $\beta$ -cells

because of the continuous insulin secretion. Thus, it seems that even under physiological conditions, a potential ER stress is present because there are many unfolded proteins and premature proteins in the ER of pancreatic  $\beta$ -cells (Harding et al 2001; Weir et al 2001). These unfolded and premature proteins can easily become targets of ROS and NO (Oyadomari et al 2001; Kaneto et al 2005). Once the ER stress is increased in pancreatic  $\beta$ -cells, the JNK- and CHOP-mediated cell death pathways are activated (Oyadomari et al 2001, 2002; Kaneto et al 2005). The activation of the JNK pathway under diabetic stress is known to induce the serine phosphorylation of insulin receptor substance 1, which in turn leads to insulin resistance (Özcan et al 2004). When the number of pancreatic  $\beta$ -cell is decreased, ER stress is increased in the remaining pancreatic  $\beta$ -cells to compensate for the reduced insulin secretion leading to pancreatic  $\beta$ -cell dysfunction (Weir et al 2001; Poitout et al 2002). Thus, ER stress-mediated pancreatic  $\beta$ -cell death is critical and a key alteration for the pathogenesis of type 2 diabetes.

## ER stress involved in vascular abnormalities in eyes with diabetic retinopathy

The loss of pericytes from the microvessels in diabetic retinas is one of the characteristic pathological changes in the early stage of diabetic retinopathy. The results of a recent study indicated that the UPR, activated by ER stress, is induced in retinal pericytes by the changes in the glucose concentration (Ikesugi et al 2006). Thus, ER stress-mediated cell death is the common pathology in the death of pancreatic  $\beta$ -cells and pericytes in diabetes.

VEGF plays important roles in the pathogenesis of diabetic retinopathy (Shweiki et al 1992; Amin et al 1997; Lu et al 1998; Ishida et al 2000; Qaum et al 2001; El-Remessy et al 2003). The expression of VEGF is increased in diabetic retinas by the high-glucose, ischemia, and hypoxia, and this leads to the development of neovascularization and/or increased vascular permeability (Shweiki et al 1992; Amin et al 1997; Lu et al 1998; Ishida et al 2000; Qaum et al 2001; El-Remessy et al 2003). Abcouwer et al showed that the glutamine deprivation-induced ER stress is associated with an up-regulation of VEGF expression in human retinal pigment epithelial cells (Abcouwer et al 2002). Roybal and colleagues (2004) suggested that the homocysteine-induced ER stress is related to the over-expression of VEGF under the control of ATF4. Hyperglycemia has been suggested to increase the intracellular homocysteine

levels in the retinal pigment epithelium cells. Thus, there is good evidence that the diabetic stress-induced ER stress is involved in vascular abnormalities such as pericyte loss and neovascularization.

## ER-stress-mediated neuronal cell death

Recent studies have shown that RGCs die at the early stage of diabetes (Barber et al 1998; Asnaghi et al 2003; Oshitari and Roy 2005). The neuronal abnormalities, such as RGC death, are irreversible and may precede the vascular abnormalities including the increased vascular permeability in diabetic retinas. This is observed even in retinas with a clinical diagnosis of non-diabetic retinopathy, however neuronal abnormalities, such as the reduction of retinal nerve fiber thickness, can be detected by optical coherence tomography even at this stage (Sugimoto et al 2005).

Guo and colleagues (1997) suggest that an upset of the  $Ca^{2+}$  homeostasis in the ER caused by mutations in presenilin-1 is associated with the neuronal cell death in Alzheimer's disease. Recently, many studies have reported that the ER stress-mediated neuronal cell death is related to the pathogenesis of various neuronal diseases in the brain and retina, eg. polyglutamine diseases (Nishito et al 2002), Parkinson's disease (Imai and Takahashi 2004), amyotrophic lateral sclerosis (Tobisawa et al 2003; Wootz et al 2004), acute brain disorders (Larner et al 2004; Tajiri et al 2004; Hayashi et al 2005), and retinal ischemia (Awai et al 2006; Shimazawa et al 2007).

Other studies have shown that the PERK-CHOP pathway, one of the ER stress-mediated pathways that is induced in ischemic retinas, is related to neuronal cell death (Awai et al 2006; Shimazawa et al 2007). It is well-known that under ischemic stress, an increase of intracellular  $Ca^{2+}$  level disturbs the ER  $Ca^{2+}$  homeostasis which in turn leads to ER stress-induced neuronal degeneration (Verkhatsky and Toescu 2003). Unfolded proteins that accumulate in the ER in ischemic retinas become targets of ROS and NO. Thus, it seems reasonable that in ischemic retinas, the ER stress-mediated cell death pathways are related to the neuronal degeneration.

We have examined the IRE1-JNK pathway to determine if it is associated with the neuronal death in ischemic retinas using the ischemia-reperfusion injury model (unpublished data). Our results suggested that the expressions of IRE1 $\alpha$  and tumor necrosis factor receptor-associated factor 2 (TRAF2) were significantly increased in the ischemic retinas compared to that in control retinas. In addition, we found that the expression of ASK1, SAPK/ERK kinase 1 (SEK1), and JNK were expressed in the same degenerating neurons

of ischemic retinas (unpublished data). Thus, not only the PERK-CHOP pathway but also the IRE1-JNK pathway is associated with neuronal death in ischemic retinas.

The exact mechanism leading to the death of RGCs has not been conclusively determined especially at the early stage of diabetic retinopathy. One possible link between vascular abnormalities and neuronal abnormalities may be the changes in the glial cells at the early stage of diabetic retinopathy (Figure 2). Glial processes make contact with both the blood vessels and neurons, and form the blood-retinal barrier (Kim et al 2006) (Figure 2). Glial cells can maintain the blood-retinal barrier by expressing VEGF, and there are many studies that have shown interactions between glial cells and neuronal cells in the retina (Fruttiger et al 1996; Rauen et al 1999; Harada et al 2000). During the onset and the progression of diabetic retinopathy, Müller cells are changed, eg, up-regulation of glial fibrillary acidic protein (GFAP) and accumulation of gamma-aminobutyric acid (Ishikawa et al 1996; Lieth et al 1998; Barber et al 2000; Rungger-Brändle et al 2000). Pannicke and colleagues (2006) suggested that glial abnormalities, eg, swelling of the cell body, may lead to the retinal edema detected in diabetic retinas. Thus, neuro-glial interactions may be involved at the onset and the progression of diabetic retinopathy, and glial changes may

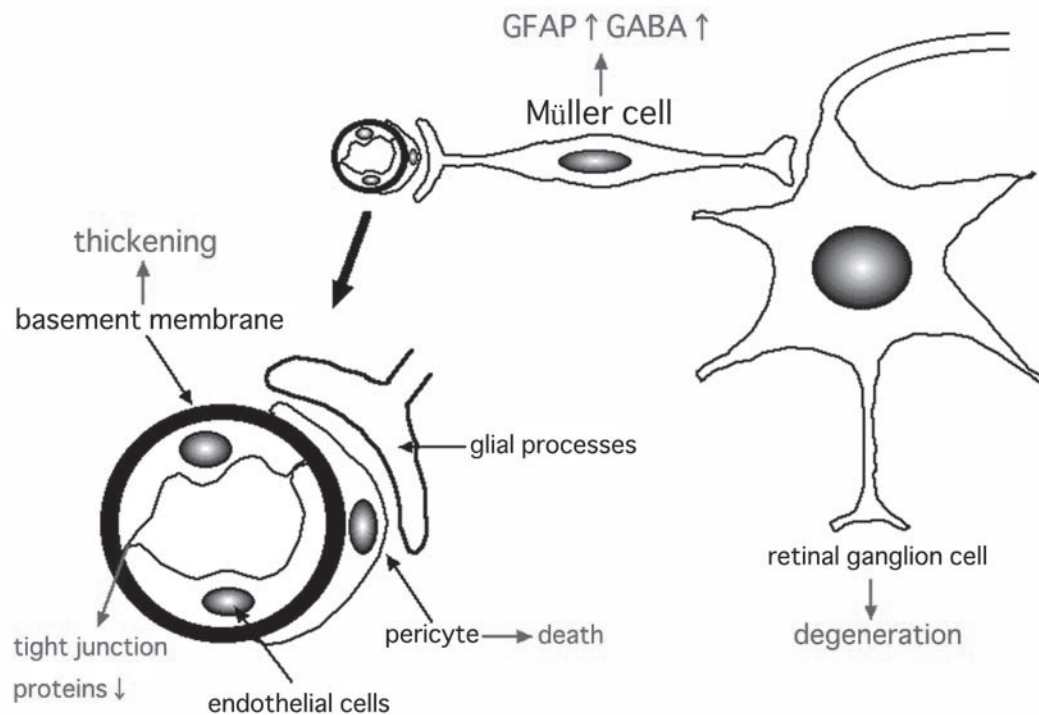
be related to both neuronal and vascular abnormalities at the early stage of diabetic retinopathy (Figure 2).

The glutamate levels are known to be elevated in the vitreous of diabetic patients (Ambati et al 1997), which could lead to neuronal cell death. Because an over-stimulation of neurons by glutamate upsets the  $Ca^{2+}$  homeostasis in the ER, ER stress may be present in degenerating neurons under diabetic stress.

Because there is very little evidence of a direct association of ER stress and the pathogenesis of diabetic retinopathy, ER stress may be an epiphenomenon and/or only one of the players, perhaps with a minor role, in the development of diabetic retinopathy. Thus, although ER-stress-related factors may be promising targets for the prevention and the progression of diabetic retinopathy, additional studies are needed to determine the relationship between ER stress and neuronal cell death in diabetic retinas.

## Potential therapeutic strategies for diabetic retinopathy

An epidemiological study performed by the Japanese Ministry of Welfare in 2005 showed that diabetic retinopathy was the second most common eye disease to cause blindness in the Japanese. Over 3,000 patients with diabetic retinopathy



**Figure 2** Hypothesized scheme of the pathogenesis of early diabetic retinopathy. The characteristic changes of early diabetic retinas may be glial changes, which in turn leads to vascular and neuronal abnormalities such as increased vascular permeability or neuronal cell death. The ideal therapies for diabetic retinopathy may be the improvement of both vascular and neuronal abnormalities.

**Abbreviations:** GFAP, glial fibrillary acidic protein; GABA, gamma-aminobutyric acid.

lose their vision each year in Japan. This indicates that the current management and therapies for diabetic retinopathy are not sufficient to prevent the progression to blindness in patients with diabetic retinopathy. To reduce the number of patients who lose their vision from diabetic retinopathy, new therapeutic strategies must be established to prevent the onset and the progression of the diabetic retinopathy.

At present, the standard treatments for diabetic retinopathy include: control of the blood glucose levels and the blood pressure (Klein et al 1995; Diabetes Control and Complications Trial Research Group 1995a, 1995b; UK Prospective Diabetes Study (UKPDS) Group 1998; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2000; Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group 2002; Matthews et al 2004), focal laser photocoagulation (Early Treatment Diabetic Retinopathy Study Research Group 1985, 1987, 1991a), pan-retinal laser photocoagulation (Diabetic Retinopathy Study Research Group 1978, 1981), and early vitrectomy (Diabetic Retinopathy Vitrectomy Study Research Group 1985a, 1985b, 1988a, 1988b, 1990). Although the diabetic retinopathy continues to progress in some patients in spite of good control of blood glucose and blood pressure, a tight control of blood glucose levels and blood pressure is the first choice for the treatment of diabetic retinopathy.

Focal laser treatment significantly decreased the risk of visual disturbances in diabetic patients with macular edema. However, side effects, eg, foveal burns, central visual field defects and retinal fibrosis, are not uncommon. Pan-retinal photocoagulation also has many side effects such as visual field constriction, night blindness, and exacerbation of macular edema. However, pan-retinal laser photocoagulation significantly decreased the risk of visual disturbances in diabetic patients with severe non-proliferative and proliferative retinopathy. Early vitrectomy reduced the risk of visual disturbances in diabetic patients with proliferative retinopathy and vitreous hemorrhage. Again, there are many side effects of vitrectomy including vitreous hemorrhage, retinal detachment, neovascular glaucoma, and infection.

Intravitreal or sub-Tenon injections of triamcinolone acetonide (TA) has been recently used to treat the macular edema common in diabetic patients, and during the early post-TA period, there were improvements in both the macular edema and visual acuity (Martidis et al 2002; Avitabile et al 2005; Sorbin and D'Amico 2005; Jonas et al 2006a). Steroids are known to up-regulate the expression of tight junction proteins, eg, occludin and ZO-1, in endothelial cells, and

this increase reduce the increased vascular permeability in the retina (Antonetti et al 2002). Unfortunately, repeated injections of TA are frequently required, and there are many side effects such as increased intraocular pressure, cataracts, and infections (Gillies et al 2004; Jonas et al 2005, 2006b; Westfall et al 2005). Although long-term follow-up studies of TA must be made, the treatments by TA may be considered together with laser treatments and vitrectomy in diabetic patients with severe macular edema.

There are many medical therapies that are being tried to prevent the development and progression of diabetic retinopathy, eg, aspirin (Early Treatment Diabetic Retinopathy Study Research Group 1991b; Chew et al 1995), anti-VEGF agents (Cunningham et al 2005; Avery et al 2006; Chun et al 2006; Spaide et al 2006), protein kinase C inhibitors (PKC-DRS Study Group 2005, 2006; PKC-DMES Study Group 2007), growth hormone inhibitors (Kirkegaard et al 1990), and aldose reductase inhibitors (Sorbinil Retinopathy Trial Research Group 1990). However, Mohamed and colleagues (2007) stated that these treatments cannot be recommended for routine use because evidence to support their use has not been published.

The most important factor to consider in the management and the treatment of diabetic retinopathy is the protection of visual function. Because the onset and progression of neuronal and vascular abnormalities lead to visual dysfunction, the targets of the therapeutic methods should be the factors that are common to both vascular and neuronal abnormalities of diabetic retinopathy. Thus, we have stated in an earlier review article that two of the candidates common to both vascular and neuronal abnormalities in diabetic retinopathy are tumor necrosis factor- $\alpha$  and Bax (Oshitari 2006). ER stress-related factors should also be considered as targets of new therapeutic strategies for diabetic retinopathy as well as the targets of new therapies for type 2 diabetes because ER stress is also related to pancreatic  $\beta$ -cell death and insulin resistance in patients with type 2 diabetes.

The results of a recent study showed that oral administration of chemical chaperones, 4-phenyl butyric acid or taurine-conjugated ursodeoxycholic acid, alleviated ER stress and improved the action of systemic insulin in diabetic animals (Özcan et al 2006). Although the precise mechanism involved in the improvement of type 2 diabetes is unclear, these chemical chaperones may stabilize protein conformation and improve the folding capacity of the ER, which in turn would reduce the ER stress in these diabetic animals. These chemical chaperones may become a standard treatment for type 2 diabetes because of their safety profiles in vivo (Maestri et al 1996; Kaplan and Gershwin 2005).



Recently, a Bax inhibitor-1 (BI-1) was identified to be an anti-apoptotic protein in mammals (Chae et al 2003), and this is relevant because BI-1 can regulate a cell death pathway involved in ER stress (Chae et al 2004). In addition, an over-expression of BI-1 can protect against the neuronal cell death induced by ER stress (Dohm et al 2006). Although the mechanism for this protective effect was not determined, a recent study showed that BI-1 can inhibit ER stress proteins such as CHOP, IRE1 $\alpha$ , or phospho-JNK (Lee et al 2007). The activity of BI-1 may provide clues on developing new ways to regulate the ER stress-induced apoptosis. For example, brain-derived neurotrophic factor (BDNF), which is known to reduce the neuronal degeneration of diabetic retinas in vivo (Seki et al 2004), prevents ER stress-mediated neuronal cell death by suppressing caspase-12 activation in vitro (Shimoke et al 2004).

Although these neuroprotective therapies may be promising, the prevention of neuronal cell death under chronic diabetic stress may have limitations. Even if one major cell death pathway is blocked, another cell death pathway may become activated. Even when apoptosis can be blocked, other types of cell death such as necrosis or autophagy may be induced (Koh et al 1995; Hartmann et al 2001; Vandenberg et al 2006). Thus, even if we can establish neuroprotective therapies for diabetic retinopathy, the first choice of the treatment for diabetic retinopathy must still be the standard treatment of controlling blood glucose levels and blood pressure to reduce the causes of diabetic stress.

In conclusion, ER stress is involved in the pathogenesis of type 2 diabetes and diabetic retinopathy. The reduction of ER stress by chemical chaperones such as 4-phenyl butyric acid or taurine-conjugated ursodeoxycholic acid may become one of the standard treatments for type 2 diabetes. Such treatments may be helpful in preventing the development of diabetic retinopathy. Additional studies are required to determine the optimal methods to reduce ER stress in patients with diabetic retinopathy.

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