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Forum Review

Endoplasmic Reticulum Stress Signaling in Pancreatic β-Cells

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ABSTRACT

Pancreatic β-cells are specialized for the production and regulated secretion of insulin to control blood-glucose levels. Increasing evidence indicates that stress-signaling pathways emanating from the endoplasmic reticulum (ER) are important in the maintenance of β-cell homeostasis. Under physiological conditions, ER stress signaling has beneficial effects on β-cells. Timely and proper activation of ER stress signaling is crucial for generating the proper amount of insulin in proportion to the need for it. In contrast, chronic and strong activation of ER stress signaling has harmful effects, leading to β-cell dysfunction and death. Therefore, to dissect the molecular mechanisms of β-cell failure and death in diabetes, it is necessary to understand the complex network of ER stress-signaling pathways. This review focuses on the function of the ER stress-signaling network in pancreatic β-cells. *Antioxid. Redox Signal.* 9, 2335–2344.

INTRODUCTION

OUR BODIES, TO FUNCTION PROPERLY, require proteins. Proteins, which are synthesized in our cells, form the basic building blocks of cells, tissues, enzymes, and organs. However, newly synthesized proteins are not immediately functional. To become functional, they must mature inside the endoplasmic reticulum (ER). In the lumen of the ER, newly produced proteins obtain their proper three-dimensional structures and mature to carry out their functions; this process is known as protein folding. The ER has an essential function in this process, especially for secreted proteins and receptors such as insulin, amyloid beta, and serotonin transporter. Defects in these three proteins cause diabetes, Alzheimer disease, and bipolar disorder, respectively.

In humans, protein folding in the ER is crucial for homeostasis and metabolism. However, the sensitive folding environment of the ER can be perturbed by pathological processes such as viral infections, environmental toxins, and mutant protein expression, as well as by physiological processes such as aging and the large biosynthetic load placed on the ER during postprandial stimulation of insulin synthesis (Fig. 1). When the demand that the load of proteins makes on the ER exceeds its folding capacity, a condition called ER stress results. Because ER stress can be elicited under physiological conditions, our cells have an adaptive response to it; this response is the unfolded protein response (UPR). As long as the UPR can deal with ER stress, our cells maintain ER homeostasis and generate the proper amount of protein in proportion to the need for it. However, when the ER stress level is too high or a defect is present in the UPR, cells cannot maintain ER homeostasis, leading to cell dysfunction and death. We classify the human diseases that are associated with high levels of chronic ER stress as ER stress diseases. They include Alzheimer's disease, Parkinson's disease, and diabetes mellitus. Diabetes, in which pancreatic β-cell death is an important component, is a good model for studying the pathogenesis of ER stress diseases. Evidence from our laboratory strongly suggests that ER stress has an important function in β-cell death during the progression of type 1 and type 2 diabetes, as well as a genetic form of diabetes, Wolfram syndrome (18, 44, 45).

Diabetes is a group of chronic disorders defined by hyperglycemia, a state of high blood glucose. This is caused by either an absolute deficiency of insulin (type 1 diabetes) or a rel-

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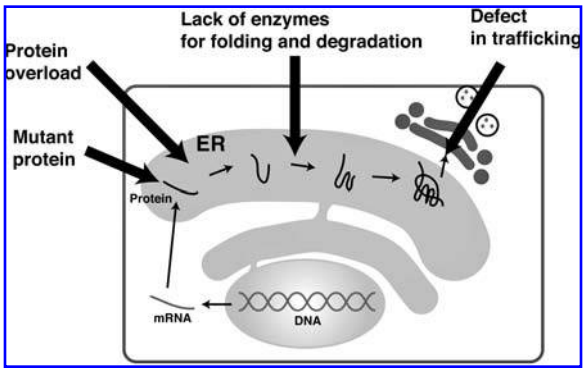

FIG. 1. ER stress. This is caused by the accumulation of misfolded proteins in the ER. Many environmental and genetic factors can cause dysregulation of ER homeostasis and thus lead to ER stress.

ative deficiency of insulin (type 2 diabetes). Insulin, a hormone secreted from β-cells in the pancreas, functions in reducing blood glucose (4). Pancreatic β-cell death is a pathogenic component in the progression of both type 1 and type 2 diabetes (7, 47). Recent observations suggest that ER stress contributes to β-cell death, autoimmunity, and insulin resistance in patients with diabetes. Here we review the physiological and pathological roles of the ER stress-signaling network in pancreatic β-cells.

ER STRESS-SIGNALING NETWORK IN PANCREATIC β-CELLS

Pancreatic β-cells are specialized for the production and regulated secretion of insulin to control blood glucose levels. In the presence of hyperglycemia, pancreatic β-cells secrete insulin from a readily available pool and activate the biosynthesis of insulin. The ER has an important function in insulin biosynthesis.

As preproinsulin, a precursor of insulin, is synthesized in the cytoplasm with a signal peptide, it is cotranslationally translocated into the lumen of the ER through interactions between the signal peptide and a signal-recognition particle on the ER membrane. The signal peptide of preproinsulin is then cleaved in the ER, and proinsulin is produced. In the lumen of the ER, proinsulin undergoes meticulous protein folding whereby three disulfide bonds are formed. Properly folded proinsulin is then delivered to the Golgi apparatus and packaged into secretory granules. The conversion of proinsulin to insulin takes place in the secretory granules. Mature insulin is then released by exocytosis (62, 63). The frequent fluctuation of blood glucose levels in humans requires that β-cells control proinsulin folding with exquisite sensitivity. Any imbalance between the physiological load of insulin translation into the ER and the folding capacity of the ER negatively affects the homeostasis of β-cells and leads to ER stress.

ER stress is a specific type of intracellular stress caused by the accumulation of misfolded or unfolded proteins in the ER (19, 68). The unfolded protein response (UPR) a cellular adaptive response that mitigates ER stress (19, 35, 49), has four components to attenuate stress: upregulation of molecular chaperone genes, translational attenuation, ER biogenesis, and ER-associated protein degradation (the ERAD system).

It has been demonstrated that inositol requiring 1 (IRE1), an ER-resident transmembrane protein kinase, is an upstream component of the UPR and a central regulator of UPR-specific downstream gene expression and apoptosis (8, 76, 77). The presence of abnormal proteins in the ER causes dimerization, trans-autophosphorylation, and consequent activation of IRE1. The activated IRE1 then splices X-box binding protein-1 (XBP-1) mRNA. This, in turn, leads to synthesis of the active transcription factor XBP-1 and upregulation of UPR genes, including ERAD genes such as ER-degradation–enhancing α-mannosidase-like protein and genes used for protein folding such as protein disulfide isomerase-P5 (8, 42, 85) (Fig. 2). XBP-1 signaling is also important for expansion of the ER (69, 71). Expansion of the ER *via* IRE1-XBP-1 signaling is particularly important for the differentiation of secretory cells such as antibody-secreting plasma cells (61) and, most likely, pancreatic β-cells. If the overload of unfolded proteins is not resolved *via* IRE1 signaling, prolonged ER stress activates the cell-death pathway.

Under chronic ER stress, IRE1 recruits TNF-receptor–associated factor 2 (TRAF2) (77), which activates apoptosis-signaling kinase 1 (ASK1) (27, 53). Activated ASK1 leads to the activation of c-Jun N-terminal protein kinase (JNK) and, in the presence of extreme ER stress, induces apoptosis in neuronal cells (52) (Fig. 2).

The mechanism underlying apoptosis *via* IRE1-JNK signaling has not yet been identified. Proteins of the BCL-2 family

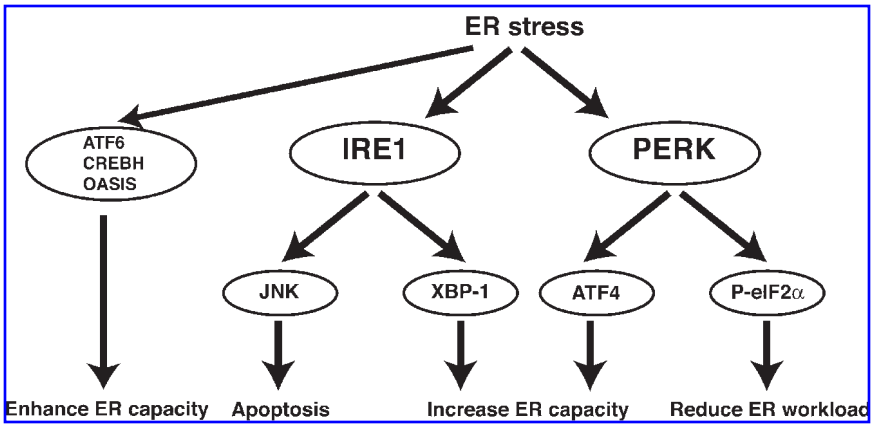

FIG. 2. ER stress-signaling network. IRE1 and PERK are transmembrane protein kinases localized to the ER membrane and the most upstream components of ER stress signaling. ER-localized transcription factors, ATF6, OASIS, and CREBH, have important functions in ER stress signaling.

are a possible link between ER stress–mediated JNK activation and apoptosis. It has been established that JNK can phosphorylate Bcl2 and Bcl-X_L, inhibiting their antiapoptotic functions (17, 37, 48, 82). It has also been reported that the proapoptotic BCL-2 family proteins, Bax and Bak, are required for JNK-dependent apoptosis (43). Although some studies have indicated that JNK activation contributes to cytokine-induced β-cell death (5), the function of ER stress–mediated JNK activation in the death of β-cells has not been studied extensively and must be defined. In addition, TRAF2 recruitment by IRE1 causes clustering of caspase-12 at the ER membrane and activates caspase-12 (84). Activated caspase-12 induces apoptosis under pathological ER stress conditions (50). The role of caspase-12 in β-cell death remains unclear.

Mammalian cells have two types of IRE1, α and β. IRE1α is ubiquitously expressed, with high levels of expression occurring in the pancreas and placenta (74). IRE1β is expressed only in epithelial cells of the gastrointestinal tract (3, 79). It has been shown that IRE1α signaling is particularly important in cells that are active in protein secretion, such as antibody-secreting plasma cells (8, 31, 32, 61).

We recently found that IRE1α activity is higher in pancreatic β-cells than it is in other tissues and that IRE1 signaling has a major function in insulin biosynthesis (44). Postprandial hyperglycemia activates IRE1α, leading to the enhancement of proinsulin biosynthesis, whereas inactivation of IRE1α signaling by siRNA hinders proinsulin biosynthesis. During insulin biosynthesis, downstream components of ER stress signaling such as BiP and ERO1α are upregulated in mouse islets. These findings indicate that IRE1α signaling is a positive regulator of insulin biosynthesis (Fig. 3). Because the activation of IRE1 by transient exposure to high glucose does not accompany XBP-1 splicing or JNK activation, we have named this unique biologic phenomenon "stimulus-coupling adaptation to ER folding (SCAEF)." The components of SCAEF probably have important functions in proinsulin biosynthesis in pancreatic β-cells. For example, an ER-resident oxidoreductase, ERO1α, is upregulated by transient high glucose and functions in proinsulin folding (K. Lipson and F. Urano, unpublished data). Because ERO1α is an activator of PDI, which is crucial in disulfide bond formation (75), ERO1α may activate insulin biosynthesis by enhancing disulfide bond formation of proinsulin in the ER. Future studies may benefit greatly from the use of activators and components of SCAEF. These may help reveal different and presently unknown UPR pathways that are not evident through the manipulation of cells with severe ER-stress–inducing drugs such as tunicamycin and thapsigargin.

PKR-like ER kinase (PERK) is another upstream component of ER stress signaling (23). Like IRE1, PERK is a transmembrane protein kinase localized to the ER membrane and is a sensor of unfolded or misfolded proteins. PERK is highly expressed in pancreatic islets (23, 70). Activated PERK phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α), leading to the attenuation of general protein translation (Fig. 2). This reduces the ER workload and protects cells from apoptosis resulting from ER stress (22). In islets from PERK knockout mice, insulin biosynthesis stimulated by high glucose is markedly enhanced, as compared with that in control mice, indicating that PERK is needed to suppress insulin biosynthesis in response to high glucose in β-cells (21). This observation, combined with our recent results, suggests that IRE1α is a positive regulator and that PERK is a negative regulator of insulin biosynthesis in fully developed β-cells (see Fig. 3). However, it has been reported that deregulated eIF2α phosphorylation also leads to declining glucose homeostasis (67). Therefore, the derepression of proinsulin biosynthesis in PERK knockout mice may reflect a defect in glucose homeostasis.

PERK also has an important function in the development of β-cells. In global PERK knockout mice, as well as pancreas-specific and endocrine-pancreas–specific PERK knockout mice, hyperglycemia and low masses of β-cells have been seen (89). In PERK knockout mice, beginning at embryonic day 16.5, β-cell proliferation was reduced by more than twofold as compared with that in wild-type mice. Also, expression levels of the insulin gene and MafA, a key marker of β-cell differentiation, were significantly reduced in PERK knockout mice. These results indicate that PERK has important functions, not only in the maintenance of β-cell function, but also in the development of β-cells. In addition to phosphorylating eIF2α, PERK is important in enhancing activating transcription factor 4 (ATF4) expression and controls ATF4 expression at the protein translation level (20). Because ATF4 functions in mitigating both ER stress and oxidative stress (24), PERK, given its control over ATF4, may have a function in protecting β-cells from ER- and oxidative-stress–mediated cell death. Mutations in the *EIF2AK3* gene encoding PERK have been reported in Wolcott–Rallison syndrome, a rare form of juvenile diabetes in humans (15). Thus, the balance between IRE1α and PERK signaling appears to be important in the maintenance of β-cell homeostasis. Indeed, an imbalance between these two pathways may cause β-cell death and diabetes.

Three other transcription factors, ATF6, OASIS, and CREBH, have important functions in ER stress signaling (26, 39, 88). All of these are bZIP-containing transcription factors localized to the ER membrane. Under ER stress, these transcription factors are cleaved and released from the ER. The bZIP domain of ATF6, OASIS, and CREBH then translocates into the nucleus and upregulates the UPR-specific downstream genes. The physiological functions of ATF6, OASIS, and CREBH in pancreatic β-cells are not yet known.

GENETIC FORMS OF DIABETES CAUSED BY ER STRESS

Wolcott-Rallison syndrome caused by mutations in the EIF2AK3 gene encoding PERK

The relationship between ER stress and diabetes was first revealed in a rare autosomal recessive form of juvenile diabetes,

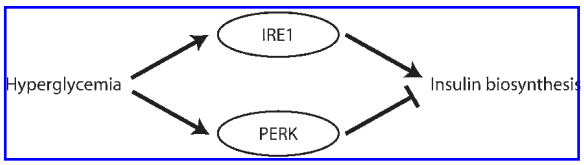

FIG. 3. IRE1 and PERK in β-cells. In pancreatic β-cells, IRE1 is a positive regulator, and PERK is a negative regulator of insulin biosynthesis.

Wolcott–Rallison syndrome. In 1972, Wolcott and Rallison (80) described two brothers and a sister with infancy-onset diabetes mellitus and multiple epiphyseal dysplasia. In this syndrome, mutations have been reported in the *EIF2AK3* gene encoding PERK, one of the most important upstream components of ER stress signaling (15) (Fig. 2). Because these mutations are within the catalytic domain of PERK, it is highly likely that they cause loss of function of the kinase activity of PERK and lead to decreased phosphorylation of eIF2α, a substrate of PERK. When a high workload is placed on the ER, eIF2α phosphorylation is essential to mitigate ER stress and thereby promote cell survival (22). Therefore, the loss of function of PERK and decreased eIF2α phosphorylation could directly lead to β-cell death. Indeed, PERK knockout mice develop diabetes because of β-cell apoptosis caused by a high level of ER stress in the pancreatic islets (21). In addition, when mutant mice with a heterozygous mutation in the phosphorylation site of eIF2α are fed a high-fat diet, they become obese and, because of β-cell dysfunction, diabetic (66). These observations strongly suggest that β-cell death in patients with Wolcott–Rallison syndrome is caused by a high level of ER stress and a defect in the UPR (*i.e.*, PERK signaling) (Fig. 4).

The negative regulator of PERK signaling, P58^{IPK}, also functions in maintaining ER homeostasis in pancreatic β-cells. P58^{IPK} is an important component of a negative-feedback loop used by these cells to inhibit eIF2-α signaling and attenuate the UPR (83). P58^{IPK} knockout mice show a gradual onset of glucosuria and hyperglycemia associated with increased apoptosis of islet cells (41). P58^{IPK} may participate in the pathogenesis of human diabetes.

Diabetes in Wolfram syndrome: the result of a high level of ER stress in pancreatic β-cells

ER stress is also a determinant of β-cell death in patients with Wolfram syndrome. In 1938, Wolfram and Wagener (81) analyzed four siblings with the combination of juvenile diabetes and optic atrophy, thus providing the first report of Wolfram syndrome. Because in a significant portion of patients with Wolfram syndrome, diabetes insipidus and auditory nerve deafness develop, this syndrome is also referred to as the diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD) syndrome (2, 59). Although patients with Wolfram syndrome are not generally obese and do not have insulitis, the β-cells in their pancreatic islets are selectively destroyed (33). Families that exhibit Wolfram syndrome share mutations in a gene encoding WFS1 protein, a transmembrane protein in the ER (29, 73). WFS1 may be an ER calcium channel (54), suggesting that this molecule has a function in ER homeostasis. However, the precise molecular mechanisms underlying β-cell death caused by the WFS1 mutation remain unknown.

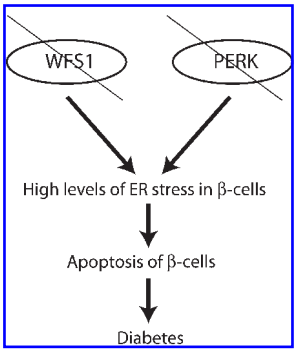

FIG. 4. A high level of ER stress in pancreatic β-cells leads to diabetes. PERK and WFS1 are necessary to mitigate ER stress in human β-cells. Therefore, the loss-of-function of PERK in Wolcott–Rallison syndrome and the loss-of-function of WFS1 in Wolfram syndrome cause high levels of ER stress in β-cells and lead to diabetes.

We recently found that WFS1 is a component of the UPR and that it functions in mitigating ER stress in pancreatic β-cells (18). We also found that WFS1 mRNA and protein are induced by ER stress and that expression of WFS1 is regulated by IRE1 and PERK. WFS1 is normally upregulated during insulin secretion, whereas the inactivation of WFS1 in β-cells causes a high level of ER stress and, consequently, β-cell dysfunction. The suppression of WFS1 causes an imbalance in ER homeostasis and leads to an increase in the expression of the ER stress markers BiP, Ero1α, spliced XBP-1, and total XBP-1 in INS-1 832/13 cells. This suppression also increases the expression of another ER stress marker, CHOP.

Recently, we also found that WFS1 negatively regulates the function of XBP-1 and ATF6 (Fonseca S and Urano F, unpublished data). This finding indicates that WFS1 has an important function in mitigating high levels of ER stress and maintaining ER homeostasis in pancreatic β-cells, suggesting that β-cell death in Wolfram syndrome is caused by chronic and high levels of ER stress. WFS1 knockout mice also develop diabetes due to β-cell apoptosis (30, 64) induced by ER stress. These results indicate that the pathogenesis of Wolfram syndrome involves chronic ER stress in pancreatic β-cells caused by the loss of function of WFS1 (see Fig. 4).

Akita mouse: diabetes mouse model caused by ER stress–mediated β-cell death

Because recent findings demonstrate the effects of ER stress in the pathogenesis of diabetes, the availability of an animal model for ER stress–mediated diabetes has become important. Recent publications indicate that ER stress leads to β-cell death in the Akita mouse, one model of diabetes (1, 36, 55, 86).

The Akita diabetes mouse model is a C57BL/6 mouse that is heterozygous for a mutation in the insulin 2 gene (78), which results in a cysteine^{96}-to-tyrosine substitution. Cysteine^{96} is involved in the formation of one of the two disulfide bonds between the A and B chains of mature insulin (46). It is highly likely that this mutation causes incorrect folding of proinsulin precursor in the ER of pancreatic β-cells and thus leads to diabetes (36, 86). Studies of the Akita diabetes model support the hypothesis that sufficient ER stress causes β-cell death (55). Therefore, the Akita mouse model is important for use in studies of the role of ER stress in β-cell death.

ER stress and type 1A diabetes (i.e., autoimmune diabetes)

Recent studies have focused on the importance of ER stress in the pathogenesis of type 2 diabetes and genetic forms of type

1 diabetes such as Wolfram syndrome. However, ER stress–mediated β-cell death may also contribute to the pathogenesis of autoimmune diabetes. Because the baseline level of ER stress is high in pancreatic β-cells, it is possible that a slight additional increase in that stress by environmental or genetic factors leads to β-cell death in patients who are genetically susceptible to ER stress. β-Cells that die as a consequence of a high level of ER stress may contain proteins with abnormal conformations (*i.e.*, misfolded proteins). The engulfment of ER stress–induced apoptotic β-cells by dendritic cells in the islets may stimulate the maturation of β-cell–reactive T cells in draining lymph nodes and lead to T-cell–mediated autoimmune destruction by "neo-autoantigens" derived from misfolded proteins (11).

Nitric oxide (NO) and ER stress in type 1 diabetes

Nitric oxide (NO) has an important function in β-cell death in type 1 diabetes (16). NO is induced by interleukin-1β (IL-1β) in combination with γ-interferon (IFN-γ) in pancreatic β-cells, leading to β-cell failure and apoptosis in type 1 diabetes. It has been shown that NO-induced β-cell apoptosis is mediated by ER stress (56). NO production decreases expression of the sarcoendoplasmic reticulum pump Ca^{2+} ATPase 2b (SERCA2b), leading to a decrease in Ca^{2+} in the ER. This Ca^{2+} depletion causes a high level of ER stress in β-cells and induces CHOP, a proapoptotic transcription factor of ER stress signaling, leading to β-cell failure and apoptosis (9, 10). Therefore, β-cell death mediated by the inflammatory cytokines IL-1β and IFN-γ is partially caused by ER stress (Fig. 5).

It has been suggested that two components of ER stress signaling contribute to β-cell death in type 1 diabetes. Activating transcription factor 3 (ATF3), a member of the ATF/CREB family of transcription factors, may have proapoptotic effects on pancreatic β-cells. In type 1 diabetes, ATF3 is induced in β-cells by proinflammatory cytokines and NO, inducers of β-cell death. Islets from ATF3 knockout mice are partially protected from cytokine- or NO-induced apoptosis; in contrast, overexpression of ATF3 in mouse islets causes dysfunction in these cells (25). These findings suggest that ATF3 is a regulator of β-cell death in the pathogenesis of type 1 diabetes (see Fig. 4).

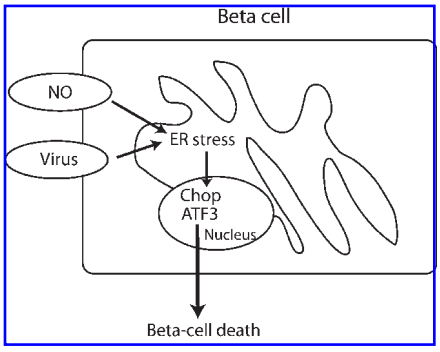

FIG. 5. ER stress and type 1 diabetes. NO, virus infection, and environmental factors can cause a high level of ER stress in β-cells, leading to β-cell death through ATF3 and CHOP signaling.

CHOP, a proapoptotic factor in ER stress signaling, also may contribute to the pathogenesis of type 1 diabetes. It has been shown that CHOP is induced by an NO donor, *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP), which increases cytosolic Ca^{2+} and induces apoptosis. Pancreatic islets from CHOP knockout mice are resistant to NO (56). Thus, CHOP may control β-cell death in type 1 diabetes (see Fig. 5).

ER stress and type 2 diabetes

Some studies suggest that a decrease in β-cell mass by apoptosis contributes to the pathogenesis of type 2 diabetes (7). The major characteristic of type 2 diabetes is peripheral resistance to the action of insulin; this resistance leads to a prolonged increase in insulin biosynthesis. Because the folding capacity of the β-cell ER is then overwhelmed, peripheral resistance to insulin may activate ER stress-signaling pathways. For this reason, chronic ER stress in β-cells may lead to β-cell death in patients with type 2 diabetes who are genetically susceptible to ER stress or have a defect in ER stress signaling (*i.e.*, UPR). Therefore, β-cell death in Wolcott–Rallison syndrome and Wolfram syndrome might be an accelerated form of the death of β-cells in patients with type 2 diabetes.

It has been suggested that three signaling components of ER stress, IRE1-JNK, CHOP, and GSK3β, have important roles in β-cell death mediated by ER stress. It has been shown that in the presence of chronic ER stress, IRE1 activates JNK through ASK1 and elicits apoptosis (52, 77). This pathway may block the functions of the antiapoptotic BCL2 family members Bcl2 and Bcl-X_L by phosphorylating them, thus causing apoptosis in β-cells. It also is possible that this pathway enhances the functions of two proapoptotic factors, Bax and Bak, and causes apoptosis. Our recent findings suggest that a transient increase in insulin biosynthesis activates IRE1 signaling in β-cells (44). Therefore, a prolonged increase in insulin biosynthesis, such as occurs in patients with insulin resistance, may elicit apoptosis through this pathway.

Another important component of β-cell death mediated by ER stress is CHOP, a member of the C/EBP family of transcription factors (65). CHOP is involved in ER stress-mediated apoptosis in fibroblasts and proximal tubule epithelial cells in the kidney (90). Recent reports suggest that CHOP also contributes to ER stress-mediated apoptosis in pancreatic β-cells (55, 56). Thus, CHOP may be a component that promotes β-cell death in patients with type 2 diabetes.

An additional component of ER stress-mediated β-cell death is glycogen synthase kinase 3β (GSK3β), a substrate of prosurvival kinase Akt (14). It has been shown that ER stress-mediated β-cell death is mediated by dephosphorylation of GSK3β as a result of attenuated Akt phosphorylation (72). Therefore, Akt and GSK3β may be potential targets for drugs to promote β-cell survival (Fig. 6).

Recent results indicate that, in addition to hyperglycemia, free fatty acids (FFAs) have important functions in inducing β-cell apoptosis (12, 34, 38). Treatment of insulinoma cells with palmitate increases expression levels of ER stress markers such as ATF4 and XBP-1 splicing. Because FFA has been shown to contribute to β-cell death in an obesity-associated diabetes model, FFA-mediated ER stress could be an important pathogenic component of type 2 diabetes (13).

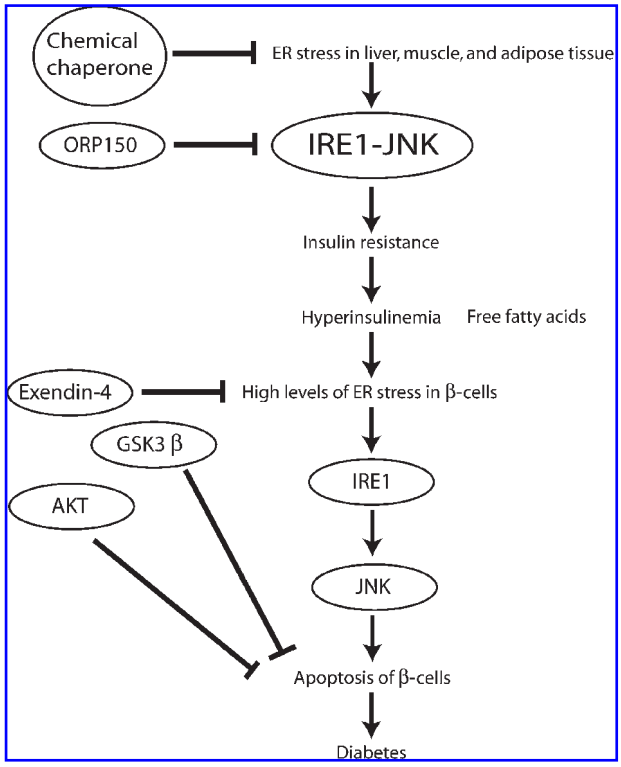

FIG. 6. ER stress and type 2 diabetes. A high level of ER stress in peripheral tissues caused by obesity, and genetic factors can lead to insulin resistance through hyperactivation of JNK signaling. Further, insulin resistance can lead to β-cell exhaustion mediated by IRE1-JNK signaling. Chemical chaperones, exendin-4, ORP-150, and GSK3 beta can reduce ER stress and might be used as novel diabetes drugs.

ER stress may be also involved in insulin resistance in peripheral tissues such as liver, muscle, and fat. It has been suggested that activation of IRE1-JNK signaling is an important contributor to insulin resistance in the liver cells of patients with type 2 diabetes. Obesity causes ER stress in the liver and leads to hyperactivation of JNK signaling (57). This, in turn, causes serine phosphorylation of insulin receptor substrate-1 (IRS-1) and inhibits insulin action in liver cells. Therefore, a high level of ER stress in liver cells contributes to the development of insulin resistance in type 2 diabetes (Fig. 6). Although it is not clear what causes ER stress in the liver, one possibility is that the baseline level of ER stress is high in hepatocytes, as it is in pancreatic β-cells, and that those cells are susceptible to additional ER stress or other types of stress. Indeed, we recently demonstrated that the baseline level of ER stress is higher in liver tissue than it is in other tissues (Ghosh R and Urano F, unpublished data). It has been shown that gene targeting of XBP-1, an important component of IRE1 signaling, causes embryonic death due to a defect in liver development in mice (60). XBP-1 knockout mouse hepatocytes fail to upregulate α-fetoprotein, an important protein in dividing hepatocytes. Therefore, ER stress signaling is important in the liver. The environmental and genetic factors that cause additional ER stress must be identified.

The insulin resistance caused by ER stress in liver cells can be reduced by overexpressing a component of the UPR, oxygen-regulated protein 150 (ORP150). Expression of ORP150, a molecular chaperone localized to the ER, is increased by ER stress (28, 40). ORP150 overexpression in liver cells can mitigate insulin resistance in db/db mice, a mouse model of type 2 diabetes (51), whereas suppression of ORP150 in liver cells decreases insulin sensitivity in C57Bl6 mice. These results suggest that upregulation of molecular chaperones in the ER (*i.e.*, components of the UPR) mitigate insulin resistance in liver cells. ORP150 and other components of the UPR could be targets for therapy against insulin resistance and type 2 diabetes (Fig. 6).

NEW APPROACHES TO DIABETES TREATMENT WITH DRUGS THAT MITIGATE ER STRESS IN PANCREATIC β-CELLS

Increasing evidence indicates a strong link between ER stress and the pathogenesis of diabetes. In both type 1 and type 2 diabetes, pancreatic β-cell death caused by a high level of ER stress is an important causative factor. Therefore, drugs that mitigate pathological ER stress in β-cells could be used to treat both types 1 and 2 diabetes. It has been shown that activation of glucagon-like peptide-1 receptor (GLP-1R) improves β-cell survival by mitigating ER stress (87). Treatment of islets with a GLP-1R agonist, exendin-4, an FDA-approved drug for diabetes treatment, reduces ER stress in a diabetes mouse model. Exendin-4 attenuates translational downregulation of insulin, induces ATF4, and improves β-cell survival. Because ATF4 is a component of PERK signaling, these results suggest that exendin-4 protects β-cells from ER stress-mediated apoptosis by activating PERK signaling (see Fig. 6).

Other compounds that can modulate a high level of ER stress in islets could be used to treat diabetes. However, it should be

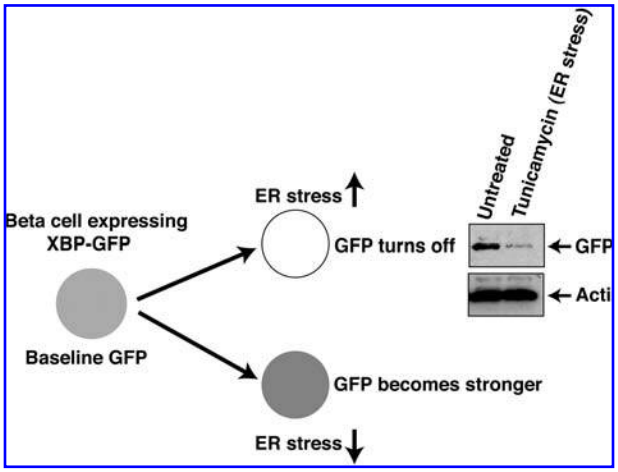

FIG. 7. Pancreatic β-cells expressing XBP1-GFP fusion protein will be used in determining the ability of chemical compounds to modulate ER stress. In this system, when the cellular ER stress level is high, the GFP signal turns off. When cellular ER stress is mitigated, the GFP signal becomes stronger.

noted that careful examination of each compound is necessary to achieve therapeutic efficacy without ceding safety. For example, a selective inhibitor of eIF2α dephosphorylation, salubrinal, enhances eIF2α phosphorylation. Because eIF2α phosphorylation is an important prosurvival component of ER stress signaling, salubrinal usually protects cells from ER stress-mediated apoptosis (6). However, treatment of β-cells with salburinal enhances FFA-mediated β-cell death (12). This unexpected phenomenon probably occurs because salubrinal treatment leads to upregulation of CHOP, a proapoptotic gene. Because the basal activation level of ER stress signaling is higher in β-cells than it is in other tissues, special precautions must be taken in testing compounds that can modulate ER stress signaling.

Drugs that can modulate ER stress in tissues other than those involved in diabetes also could be used to treat diabetes. Chemical compounds that mitigate ER stress in peripheral tissues have been shown to restore glucose homeostasis in a mouse model of type 2 diabetes (58). Two chemical chaperones, 4-phenyl butyric acid (PBA) and taurine-conjugated ursodeoxycholic acid, alleviate ER stress in the liver, resulting in the normalization of hyperglycemia and restoration of systemic insulin sensitivity in the liver, muscle, and adipose tissue. This indicates that the chemical chaperones that mitigate ER stress might be used to treat type 2 diabetes (see Fig. 6).

To develop a new treatment for diabetes, it is important to identify novel compounds that can modulate ER stress levels in β-cells. Our group established a method for monitoring ER stress levels by using XBP-1-GFP fusion protein. With this method, we plan to screen the ability of different chemical compounds to modulate ER stress levels in β-cells. Effective chemical compounds identified in this screening may then be used to treat diabetes by leveraging a natural process, a prosurvival arm of ER stress signaling.

We established several INS-1 cell lines stably expressing XBP-1-GFP (INS-$1^{XBP\text{-}GFP}$). Because INS-1 cells constantly secrete insulin, a weak baseline GFP signal is found in these cells (*i.e.*, baseline splicing of XBP-1). In this system, when the cellular ER stress level is high, the GFP signal turns off because of XBP-1 splicing by ER stress. When cellular ER stress is mitigated, the GFP signal becomes stronger (Fig. 7). We are in the process of screening a chemical compound library and an shRNA library to identify drugs and genes having the ability to modify XBP-1 splicing (*i.e.*, ER stress levels). The drugs and molecules identified in these screens could lead to the development of novel treatments for diabetes.

CONCLUDING REMARKS

Diabetes, in which pancreatic β-cell dysfunction and death is an important component, is a good model for studying the pathogenesis of ER stress diseases. Evidence from our laboratory strongly suggests that ER stress has an important function in β-cell dysfunction and death during the progression of type 1, type 2, and the genetic form of diabetes, Wolfram syndrome. Therefore, to dissect the molecular mechanisms of β-cell failure and death in diabetes, it is necessary to understand the complex network of ER stress-signaling pathways.

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ABBREVIATIONS

ASK1, apoptosis-signaling kinase 1; ATF3, activating transcription factor 3; ATF4, activating transcription factor 4; DIDMOAD syndrome, diabetes insipidus, diabetes mellitus, optic atrophy, and deafness syndrome; eIF2α, α subunit of eukaryotic translation initiation factor 2; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum–associated protein degradation; FFAs, free fatty acids; GLP-1R, glucagon-like peptide-1 receptor; GSK3β, glycogen synthase kinase 3β; IFN-γ, γ-interferon; IL-1β, interleukin-1β; IRE1, inositol requiring 1; IRS-1, insulin receptor substrate-1; JNK, c-Jun N-terminal protein kinase; NO, nitric oxide; ORP150, oxygen-regulated protein 150; PBA, 4-phenyl butyric acid. PERK, PKR-like ER kinase; SCAEF, stimulus-coupling adaptation to ER folding; SERCA2b, sarcoendoplasmic reticulum pump Ca^{2+} ATPase 2b; SNAP, *S*-nitroso-*N*-acetyl-D,L-penicillamine; TRAF2, TNF-receptor–associated factor 2; UPR, unfolded protein response; XBP-1, X-box binding protein-1.

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