

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Endoplasmic Reticulum (ER) Stress in the Pathogenesis of Type 1 Diabetes

Jixin Zhong

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52644>

1. Introduction

As one of the major health problems in the world, diabetes affects over 346 million people worldwide. In United States alone, according to the statistical fact sheet released 2011 by American Diabetes Association, 25.8 million children and adults accounting for 8.3% of the population are affected by diabetes. Unfortunately, the therapy of diabetes remains unsatisfied despite of extensive studies in the last decades. Diabetes can be categorized into two main types: type 1 and type 2. Type 1 diabetes mellitus, used to known as juvenile diabetes, is typically developed in children and juveniles. Despite the increasing rate of Type 2 diabetes in the United States, type 1 diabetes accounts for over 2/3 of new adolescent diabetes diagnoses. Although most commonly presented in childhood, type 1 diabetes also accounts for 5-10% cases of adult diabetes (1). Recent epidemiologic studies revealed that the incidence for type 1 diabetes in most regions of the world has increased by 2-5% (2).

Unlike type 2 diabetes, which is caused by the loss of insulin sensitivity, type 1 diabetes is caused by insulin deficiency following destruction of insulin-producing pancreatic β cells. Autoimmune-mediated β cell death has been considered as the major cause of β -cell loss in type 1 diabetes. However, the underlying mechanisms are not fully understood. Accumulating evidence suggests an involvement of endoplasmic reticulum (ER) stress in multiple biological processes during the development of type 1 diabetes. Pancreatic β cells exhibit exquisite sensitivity to ER stress due to their high development in order to secrete large amounts of insulin. There is also evidence supporting that ER stress regulates the immune cell functionality and cytokine production that is relevant to autoimmune processes in type 1 diabetes. Furthermore, β cell loss caused by autoimmune attack results in an increased ER burden on the rest pancreatic β cells and induces unfolded protein response (UPR) and ER stress, which further exacerbates β cell death. Here I will

summarize the functional involvement of ER stress in the pathogenesis of type 1 diabetes and the potential underlying mechanisms.

2. Pancreatic β cell and blood glucose regulation

2.1. Blood glucose regulation by pancreas

The major cause of type 1 diabetes is loss of insulin-secreting pancreatic β cell and insulin inadequacy (3;4). For a better understanding of the pathogenesis of type 1 diabetes, the regulatory mechanisms of blood glucose by pancreas will be briefly introduced. Blood glucose level is closely regulated in order to provide a homeostatic microenvironment for tissues and organs. According to the American Diabetes Association, a normal fasting blood glucose level is between 70 to 100 mg/dL, and the recommended fasting level is to aim for 70 to 130 mg/dL and less than 180 mg/dL after meals (5). Blood glucose is monitored by the cells in the islets of Langerhans (6). Islets of Langerhans are clusters of pancreatic cells that execute the endocrine function of pancreas. They contain the following 4 types of cells, in order of abundance: β cells, α cells, δ cells, and γ cells. Pancreatic β cells and α cells make up about 70% and 17% of islet cells respectively, and both of them are responsible for the blood glucose regulation by producing insulin (β cells) and glucagon (α cells) (6). Pancreatic δ cells produce somatostatin which has a major inhibitory effect, including on pancreatic juice production. Pancreatic γ cells secrete pancreatic polypeptide that is responsible for reducing appetite.

Insulin and glucagon have opposite functions on glucose regulation. They keep blood glucose level in a normal range by coordinating with each other (Figure 1). After a meal, the digestive system breaks down the carbohydrates to small sugar molecules, mainly glucose. The glucose is then absorbed across the intestinal wall and travel to the circulating bloodstream. Pancreatic β cells sense increased blood glucose level by taking up glucose through GLUT2, a glucose transporter. The metabolism of glucose in β cells leads to the increase of ATP/ADP ratio, which causes the closing of ATP-sensitive potassium channels and further leads to the open of calcium channels on membrane. The resulting increase of intracellular calcium concentration promotes the secretion of insulin into circulation of blood. Circulating insulin then acts on cells in a variety of tissues including liver, muscle, and fat through interacting with insulin receptor on the cell membrane. Insulin signaling induces the translocation of glucose transporter GLUT4 to cell membrane of muscle cells and adipocytes, leading to the uptake of glucose into cells as an energy source. In addition, insulin signaling also stimulates the conversion of glucose into glycogen, a process called glycogenesis, in liver. Therefore, insulin lowers blood glucose level by promoting glycogenesis and glucose uptake by peripheral tissues (7). In contrast, a drop in blood glucose caused by starving or other situations like extreme exercise suppresses the secretion of insulin by β cells and stimulates α cells of pancreas to release glucagon. Glucagon acts on liver and promotes glucose production by the breakdown of glycogen to glucose (called glycogenolysis), resulting in the increase of blood glucose.

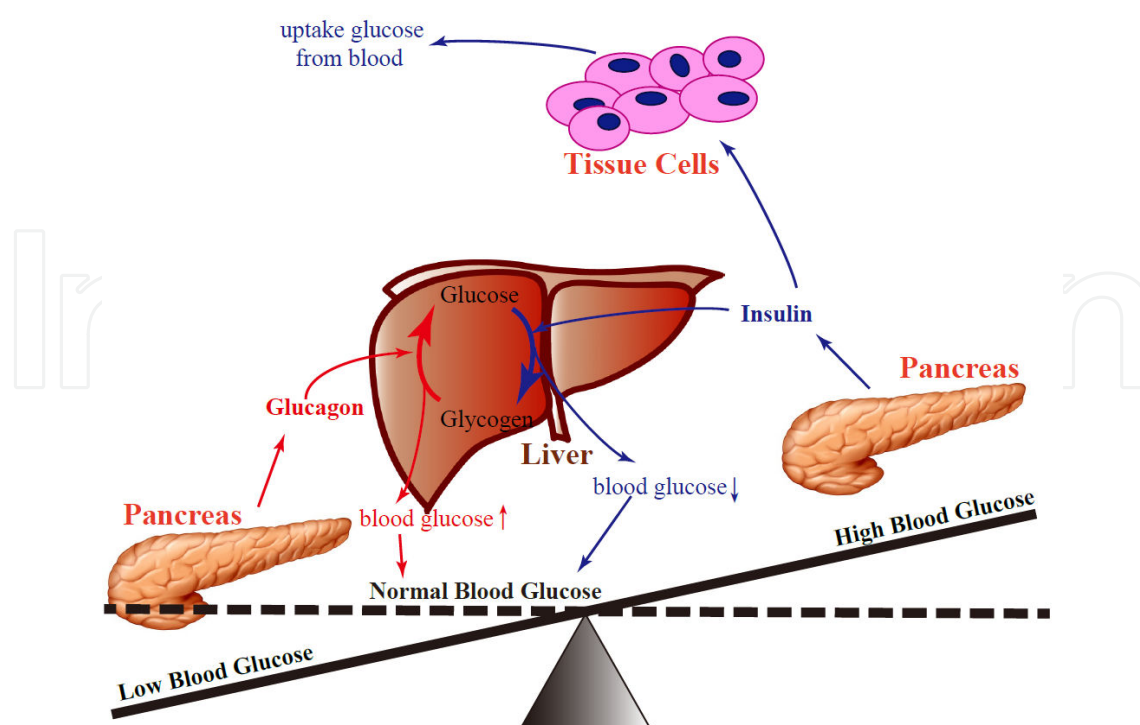


Figure 1 Homeostatic regulation of blood glucose by pancreas

Figure 1. Homeostatic regulation of blood glucose by pancreas. Pancreas is the major organ responsible for maintaining the blood glucose homeostasis. Increase of blood glucose level can be sensed by GLUT2 on β cells, a glucose transporter. The metabolism of glucose in β cells promotes the secretion of insulin into circulation of blood. Circulating insulin then increases the glucose uptake by a variety of tissues including liver, muscle, and fat. In liver, insulin signaling also stimulates the conversion of glucose into glycogen, a process called glycogenesis. Both glycogenesis and glucose uptake by peripheral tissues can lead to a decrease of glucose level in blood stream. In contrast, a drop of blood glucose level suppresses the secretion of insulin by β cells and stimulates α cells to release glucagon. Glucagon acts on liver and promotes glucose production by the breakdown of glycogen to glucose, a process called glycogenolysis, and results in the increase of blood glucose.

2.2. Pancreatic β cells and insulin biosynthesis

Either insulin deficiency or insulin inefficiency can cause diabetes. As the only cell type producing insulin, β cell plays a critical role in the development of diabetes. In type 1 diabetes, autoimmune-mediated destruction of β cell leads to insufficient insulin production and inability of cells to take up glucose. In contrast, type 2 diabetes is caused by loss of insulin sensitivity. In response to insulin resistance, the body secretes more insulin to overcome the impaired insulin action. However, pancreatic β cells fail to secrete sufficient insulin to overcome insulin resistance in some individuals, resulting in type 2 diabetes (8;9). Therefore, dysfunction of β cell exists in both types of diabetes.

Pancreatic β cell is specialized for production of insulin to control blood glucose level. In response to hyperglycemia, insulin is secreted from a readily available pool in β cells. In the meantime, the secretion of insulin activates the biosynthesis of insulin (10). Insulin is first

synthesized as preproinsulin with a signal peptide in the ribosomes of the rough endoplasmic reticulum. Preproinsulin is translocated into ER lumen by interaction of signal peptide with signal recognition particle on the ER membrane. Preproinsulin is converted to proinsulin by removing the signal peptide forming three disulfide bonds in the ER. Proinsulin is then translocated into Golgi apparatus and packaged into secretory granules that are close to the cell membrane. In the secretory granules, proinsulin is cleaved into equal amounts of insulin and C-peptide (Figure 2). Insulin is accumulated and stored in the secretory granules. When the β cell is appropriately stimulated, insulin is secreted from the cell by exocytosis (11). As the major site for protein synthesis, ER plays an important role in insulin biosynthesis. To fulfill the requirement for secreting large amount of insulin, the pancreatic β cells are equipped with highly developed ER, leading to the vulnerability of β cell to ER stress (12). In type 1 diabetes, the loss of β cell increases the burden of insulin secretion on the residual β cells. On the one hand, this compensated action is beneficial for the control of blood glucose. On the other hand, it also increases the ER burden of residual β cells, which further exacerbates β cell death.

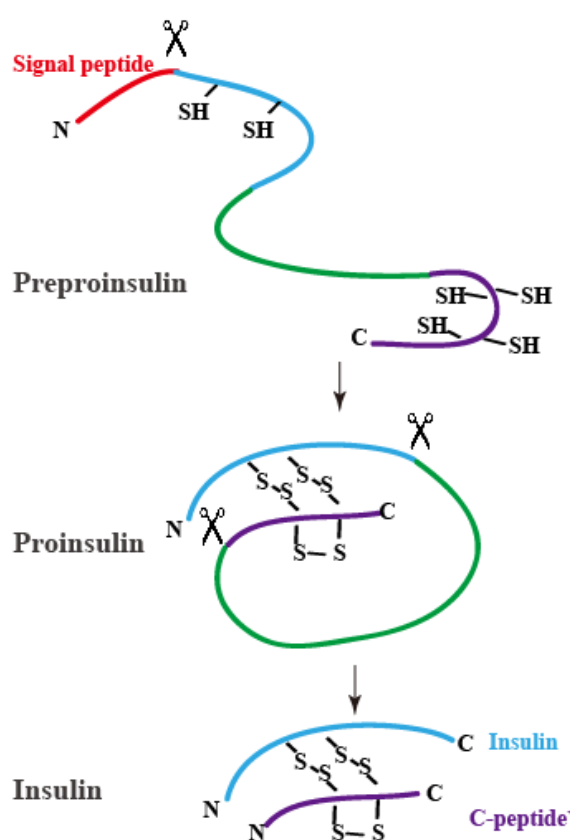


Figure 2. Biosynthesis of insulin in β cell. In the ribosomes of rough endoplasmic reticulum, insulin is first synthesized as a precursor, preproinsulin. Preproinsulin has a signal peptide that directs it to translocate into ER lumen by interacting with signal recognition particle on the ER membrane. In ER lumen, preproinsulin is converted to proinsulin by removing the signal peptide and forming three disulfide bonds. Proinsulin is then translocated into Golgi apparatus and packaged into secretory granules where it is cleaved into equal amounts of insulin and C-peptide. After synthesis, insulin is stored in the secretory granules and secreted from the cell until the β cell is appropriately stimulated.

3. Biological characterization of endoplasmic reticulum (ER) and ER stress

3.1. Endoplasmic reticulum

Endoplasmic Reticulum (ER) is an organelle of eukaryotic cells that is responsible for the facilitation of protein folding and assembly (13-15), manufacture of the membranes(16), bio-synthesis of lipid and sterol, storage of intracellular Ca^{2+} , and transport of synthesized proteins in cisternae. It is a membranous network of tubules, vesicles, and cisternae that are interconnected by the cytoskeleton. The ER is well developed in endocrine cells such as β cell in which large amounts of secretory proteins are synthesized.

ER is categorized into two types: rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). As featured by its name, RER looks bumpy and rough under a microscope due to the ribosomes on the outer surfaces of the cisternae. RER is in charge for protein synthesis. The newly synthesized proteins are folded into 3-dimensional structure in RER and sent to Golgi complex or membrane via small vesicles. In contrast, SER appears to have a smooth surface under the microscope as it does not have ribosomes on its cisternae. SER is responsible for the synthesis of lipids and steroids, regulation of calcium concentration, attachment of receptors on cell membrane proteins, and detoxification of drugs. It is found commonly in places such as in the liver and muscle. It is important for the liver to detoxify poisonous substances. Sarcoplasmic reticulum is a special type of SER. It is found in smooth and striated muscle, and is important for the regulation of calcium levels. It sequesters a large store of calcium and releases them when the muscle cell is stimulated.

3.2. Unfolded protein response and ER stress

ER stress is defined as the cellular responses to the disturbances of normal function of ER. The most common cause of ER stress is protein mis-folding. ER is the place where newly produced proteins fold into 3-dimensional conformation which is essential for their biological function. The sensitive folding environment could be disturbed by a variety of pathological insults like environmental toxins, viral infection, and inflammation. In addition to pathological insults, it can also be induced by many physiological processes such as overloaded protein biosynthesis on ER. For example, in case of type 1 diabetes, increased insulin synthesis in residual β cell exceeds the folding capacity of ER, resulting in the accumulation of unfolded insulin. The accumulation of unfolded or mis-folded proteins in the ER leads a protective pathway to restore ER function, termed as unfolded protein response (UPR).

Protein folding requires a series of ER-resident protein folding machinery. A special type of proteins called chaperones is used as a quality control mechanism in the ER. As the major mechanisms to promote protein folding, chaperones assist protein folding by interacting with the newly synthesized proteins. In addition, chaperones also help to break down unfolded or incorrectly folded proteins in the ER via a process called ER associated degradation. The monitoring mechanism ensures the correct protein folding in the ER. The unfolded proteins usually have a higher number of hydrophobic surface patches than that of proteins

with native conformation (17). Thus, unfolded proteins are prone to aggregate with each other in a crowded environment and directed to degradative pathway (18). Molecular chaperones in the ER preferentially interact with hydrophobic surface patches on unfolded proteins and create a private folding environment by preventing unfolded proteins from interaction and aggregation with other unfolded proteins. In addition, the concentration of Ca^{2+} in ER also impairs protein folding by inhibiting the activity of ER-resident chaperones and foldases (19-22). ER is the major site for Ca^{2+} storage in mammalian cells. The concentration of Ca^{2+} in ER is thousands times higher than that in the cytosol (23). Most chaperones and foldases in ER are vigorous Ca^{2+} binding proteins. Their activity, therefore, is affected by the concentration of Ca^{2+} in ER.

Exhaustion of the protein folding machineries or insufficient energy supply increases the accumulation of unfolded or mis-folded proteins in ER, which is responsible for the activation of UPR. UPR is a protective mechanism by which it monitors and maintains the homeostasis of ER. Various physiological and pathological insults such as increased protein synthesis, failure of posttranslational modifications, nutrient/glucose starvation, hypoxia, and alterations in calcium homeostasis, can result in the accumulation of unfolded or mis-folded proteins in ER which further causes ER stress (24). For example, altered expression of antithrombin III (25;26) or blood coagulation factor VIII (27;28), may result in the exhaustion of protein folding machinery and thus induces UPR. Some physiological processes such as the differentiation of B lymphocytes into plasma cells along with the development of highly specialized secretory capacity can also cause unfolded protein accumulation and activate UPR (29-31). In response to those physiological and pathological insults, cells initiate UPR process to get rid of the unfolded or mis-folded proteins. For instance, UPR can increase the folding capacity by up-regulating ER chaperones and foldases, as well as attenuate the biosynthetic burden through down-regulating the expression of secreted proteins (32-34). In addition, UPR also eliminates unfolded or mis-folded proteins by activating ER associated degradation process (35-37). However, once the stress is beyond the compensatory capacity of UPR, the cells would undergo apoptosis. As such, UPR and ER stress are reported to be implicated in a variety of pathological processes, including diabetes, neurodegenerative diseases, pathogenic infections, atherosclerosis, and ischemia (24;38).

In addition to protein folding, a variety of post-translational modifications including N-linked glycosylation, disulfide bond formation, lipidation, hydroxylation, and oligomerization, occur in ER. Disruption of those post-translational modifications can also result in the accumulation of incorrectly folded proteins and thereby induce UPR or ER stress. For example, glucose deprivation impairs the process for N-linked protein glycosylation and thus leads to ER stress (39).

3.3. ER stress pathways

As a protective mechanism during ER stress, UPR initiates a variety of process to ensure the homeostasis of ER. UPR can be mediated by three major pathways, which are initiated by the three transmembrane signaling proteins located on the ER membrane. Those transmembrane proteins function as a bridge linking cytosol and ER with their C-terminal in the cyto-

sol and N-terminal in the ER lumen. The N-terminal is usually engaged by an ER resident chaperone BiP (Grp78) to avoid aggregation. When unfolded proteins accumulate in ER, chaperons are occupied by unfolded proteins and release those transmembrane signaling proteins. There are three axes of signals that are initiated by the pancreatic endoplasmic reticulum kinase (PERK), the inositol-requiring enzyme 1 (IRE1), and the activating transcription factor 6 (ATF6) respectively. The release of these proteins from BiP triggers UPR and ER stress (Figure 3).

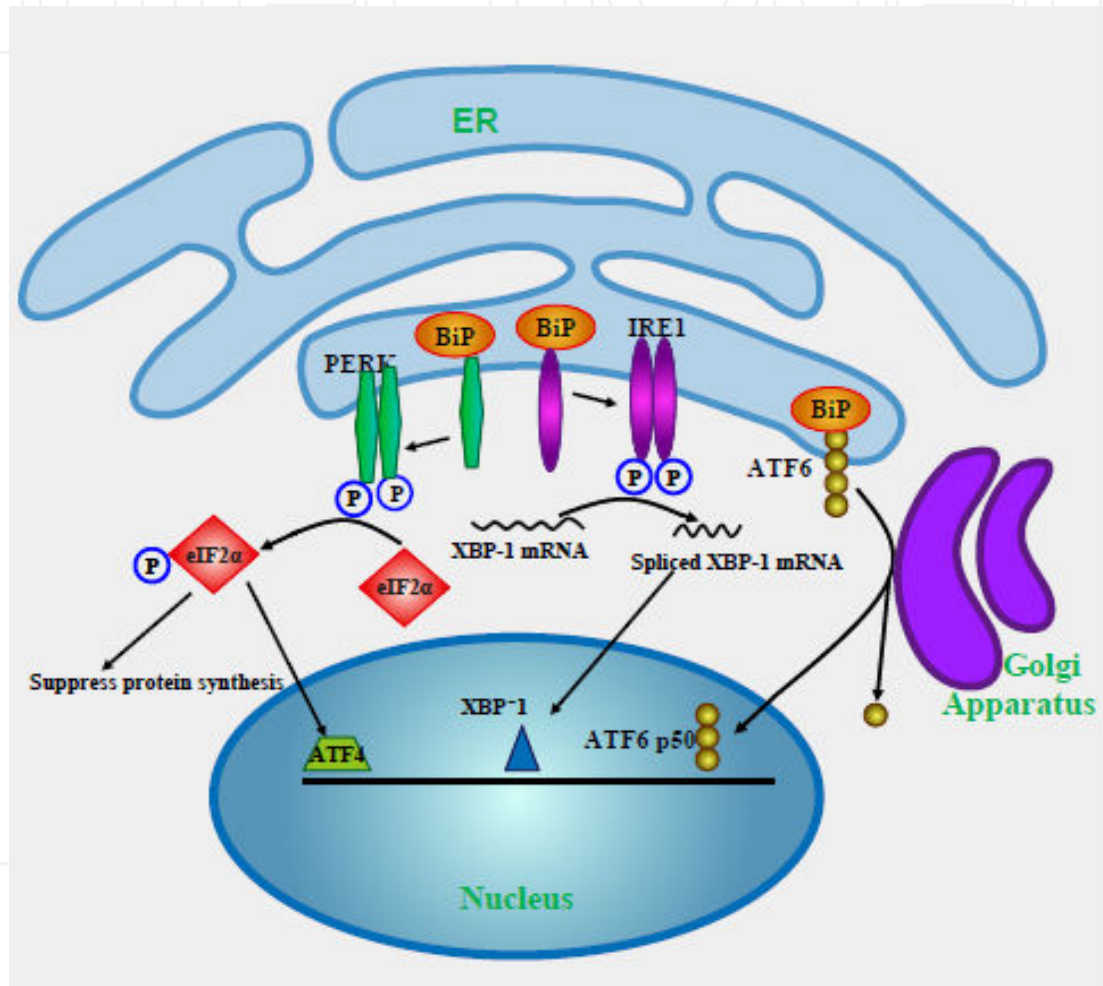


Figure 3. UPR signal pathways. Under normal condition, PERK, IRE1, and ATF6 binding to the ER chaperone BiP to remain inactive state. Upon the accumulation of unfolded proteins, BiP preferentially binds to the unfolded proteins, leading to the release of PERK, IRE1, and ATF6. PERK becomes oligomerized and activated once released from BiP, and subsequently phosphorylates eIF2α. The phosphorylation of eIF2α results in the suppression of the overall transcription of mRNAs and selectively enhanced transcription of genes implicated in UPR such as the ATF4 mRNA. Similar to PERK, IRE1 is dimerized and activated after released from BiP. Activated IRE1 induces XBP-1 by enhancing the splicing of its mRNA. XBP-1 enhances UPR by regulating the transcription of its target genes. The detachment of ATF6 from BiP results in the translocation of ATF6 to the Golgi apparatus and cleavage of ATF6. Cleaved ATF6 then translocates into the nucleus and initiates the transcription of target genes.

PERK/eIF2α/ATF4 axis: PERK is a type I transmembrane Ser/Thr protein kinase uniquely present in ER. In response to ER stress, the binding of unfolded proteins to BiP leads to the

release of PERK from BiP. Once released from BiP, PERK becomes oligomerized and auto-phosphorylated. As a result, PERK inactivates eukaryotic initiation factor 2 α (eIF2 α) by the phosphorylation of Ser51 to inhibit mRNA translation and protein load on ER (34;40). In addition, phosphorylated eIF2 α also promotes the expression of stress-induced genes including the transcription factors ATF4 and CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) (41). Deficiency of PERK results in an abnormally elevated protein synthesis in response to the accumulation of unfolded proteins in ER.

IRE1/XBP-1 axis: IRE1 is another axis of signal involved in UPR. There are 2 isoforms of IRE1: IRE1 α and IRE1 β . IRE1 α is expressed in most cells and tissues, while IRE1 β is restricted in intestinal epithelial cells (42;43). Once disassociated with BiP, IRE1 becomes activated. Activated IRE1 possesses endoribonuclease activity and cleaves 26 nucleotides from the mRNA encoding X-box binding protein-1 (XBP-1), resulting in the increased production of XBP-1 (44). XBP-1 is a transcriptional factor belonging to basic leucine zipper transcription factor family. It heterodimerizes with NF- κ B and enhances gene transcription by binding to the ER stress enhancer and unfolded protein response element in the promoters of targeted genes involved in ER expansion, protein maturation, folding and export from the ER, and degradation of mis-folded proteins (44-49). In addition, IRE1 α also mediates the degradation of ER-targeted mRNAs, thus decreasing the ER burden (50).

ATF6 axis: The third axis of ER stress signal is mediated by ATF6. Unlike PERK and IRE1 which oligomerize upon UPR, ATF6 translocates into the Golgi apparatus after released from BiP. The transmembrane domain is then cleaved in the Golgi apparatus (51). The 50-kDa cleaved ATF6 is relocated into the nucleus where it binds to the ER stress response element CCAAT(N)9CCACG to regulate the expression of targeted genes. For example, once released from the ER membrane, ATF6 enhances the transcription of XBP-1 mRNA which is further regulated by IRE1 (44). In addition, ATF6 also increases the expression of the two major chaperon systems in the ER: calnexin/calreticulin and BiP/GRP94 (44;52;53).

4. The implication of ER stress in autoimmune responses

4.1. ER stress and innate immune response

The importance of innate immunity was highlighted in the pathophysiology of type 1 diabetes (54-57). Type 1 diabetes was initially considered a T-cell-mediated autoimmune disease (58), in which T-cell was believed as the major immune cell causing β cell destruction while the involvement of innate immune response has been ignored for a long time. However, recent studies suggest a critical role of innate immune responses in the development of type 1 diabetes (54;55). As the first line of defense mechanism, innate immunity is implicated in the initiation as well as the progression of autoimmune responses against pancreatic β cell.

Innate immune response is regulated by elements of the UPR pathway (59). For example, Cyclic-AMP-responsive-element-binding protein H(CREBH), an ER stress-associated transcription factor, regulates the expression of serum amyloid P-component and C-reactive

protein, the two critical factors implicated in innate immune responses. Like ATF6, CREBH is an ER-membrane-bound protein. In response to ER stress, CREBH release an N-terminal fragment and transit to nucleus to regulate the expression of target genes. Innate immune response, in turn, regulates the expression of CREBH through inflammatory cytokines such as IL-1 β and IL-6 (60). The development of dendritic cells, the major innate immune cells, is also regulated by ER stress response (61). High levels of mRNA splicing for XBP-1 are found in dendritic cell, and mice deficient in XBP-1 show defective differentiation of dendritic cell. Both conventional (CD11b⁺ CD11c⁺) and plasmacytoid dendritic cells (B220⁺ CD11c⁺) are decreased by >50%. Dendritic cells deficient for XBP-1 are vulnerable to ER stress-induced apoptosis (61). Moreover, the secretion of inflammatory cytokine IL-23 by dendritic cell also involves ER stress response. CHOP, a UPR mediator, can directly bind to the *IL-23* gene and regulate its transcription. ER stress combined with Toll-like receptor (TLR) agonists was found to markedly increase the mRNA of IL-23 p19 subunit and the secretion of IL-23, while knockdown of CHOP suppressed the induction of IL-23 by ER stress and TLR signaling (62).

The association of ER stress with innate immune response is confirmed in many disease models. Richardson and coworkers reported that innate immune response induced by *P. aeruginosa* infection causes ER stress in *C. elegans*, and loss-of-function mutations of XBP-1 lead to larval lethality (63). In consistent with that, polymorphisms of *XBP-1* gene were found to be associated with Crohn's disease and ulcerative colitis in humans (64), the two autoimmune diseases share similar properties with type 1 diabetes. Lack of XBP-1 in intestinal epithelial cells may induce Paneth cell dysfunction which further results in impaired mucosal defense to *Listeria monocytogenes* and increased sensitivity to colitis (64).

In addition to IRE1/XBP-1 axis, PERK/eIF2 α /ATF4 axis of UPR is also associated with innate response. TLR signaling, the most important innate signaling pathway, can induce selective suppression of the PERK/eIF2 α /ATF-4/CHOP axis of UPR pathway (65). The activation of TLR decreases eIF2 α -induced ATF4 translation. For instance, pretreatment of LPS, an agonist for TLR4, attenuated ATF4/CHOP signaling and prevented systemic ER stress-induced apoptosis in macrophages, renal tubule cells, and hepatocytes (65). In contrast, loss of Toll-IL-1R-containing adaptor inducing IFN- β (TRIF), an important adapter for TLR signaling, abrogated the protective effect of LPS on renal dysfunction and hepatosteatosis induced by ER stress, suggesting that TLR signaling suppresses ATF4/CHOP via a TRIF-dependent pathway (65).

4.2. ER stress and adaptive immune response

The presence of β cell specific autoantibodies is a marker for autoimmune diabetes (66). IRE1/XBP1 axis is required for the differentiation of antibody-producing B lymphocytes. IRE1 is necessary for the Ig gene rearrangement, production of B cell receptors, and lymphopoiesis. The expression multiple UPR components including BiP, GRP94, and XBP-1 is up-

regulated during the differentiation of B cells (67). Mice with a deficiency of IRE1 in hematopoietic cells have a defective differentiation of pro-B cells towards pre-B cells (68). XBP-1, an IRE1 downstream molecule, is also involved in the differentiation of B cell and antibody production by mature B cells. It was found that the engagement of B-cell receptor induces ubiquitin-mediated degradation of BCL-6, a repressor for B-lymphocyte-induced maturation protein 1 (69), while B-lymphocyte-induced maturation protein 1 negatively regulates the expression of B-cell-lineage-specific activator protein (70), a repressor for XBP-1 (71). In line with these results, B lymphocytes deficient in B-lymphocyte-induced maturation protein 1 failed to express XBP-1 in response to LPS stimulation (72). The expression of XBP-1 is rapidly up-regulated when B cells differentiate into plasma cells. Furthermore, XBP-1 is able to initiate plasma cell differentiation when introduced into B-lineage cells. XBP-1-deficient lymphoid chimeras have a defective B-cell-dependent immune response due to the absence of immunoglobulin and plasma cells (30). In addition to IRE1/XBP-1 axis, ATF6 axis may also be implicated in the differentiation of B cells, as increased ATF6 cleavage is found in differentiating B cells (67). However, PERK axis does not seem to be involved in the B-cell differentiation and maturation (68;73).

Activation of T lymphocyte, another important adaptive immune cell, seems also involves UPR. TCR engagement, the first T cell activation signal, induces the expression of ER chaperons including BiP and GRP94. Inhibition of protein kinase C, a serine/threonine protein kinase downstream of TCR signaling, suppresses the activation of ER stress response induced by T cell activation (74). IRE1/XBP-1 axis regulates the differentiation of effector CD8⁺ T cell. IRE1/XBP-1 pathway is activated in effector CD8⁺ T cell during acute infection. IL-2 promotes XBP-1 mRNA transcription, while TCR ligation induces the splicing of XBP-1 mRNA. The differentiation of CD8⁺ T cell is reduced by suppression of XBP-1 (75). Other than IRE1/XBP-1, CHOP is also involved in the functionality of T cells. A recent report suggests GTPase of the immunity-associated protein 5 (Gimap5) mutation in BioBreeding diabetes-prone rat, a model for type 1 diabetes, leads to ER stress and thus induces spontaneous apoptosis of T cells. Inhibition of CHOP protects Gimap5^{-/-} T cells from ER stress-induced apoptosis (76).

4.3. ER stress regulates cytokine production

Cytokine production is an important inflammatory process in response to insults of pathogens, mutated self-antigens or tissue damage. ER stress is interconnected with the induction of inflammatory cytokines through multiple mechanisms including reactive oxygen species (ROS), NFκB and JNK (Figure 4). ROS are defined as highly reactive small molecules with unpaired electrons. They are important mediators of inflammatory response. Oxidative stress, caused by the accumulation of ROS, was confirmed to be associated with ER stress (77). For example, the disulphide bond formation during the process of protein folding requires oxidizing condition (78). Therefore, increased protein folding load may lead to oxidative stress. The PERK axis of UPR is able to activate antioxidant pathway by promoting ATF4 and nuclear factor-erythroid-derived 2-related fac-

tor 2 (NRF2) (79;80). Therefore, deficiency of PERK markedly increases ROS accumulation in response to toxic chemicals (79;81). The IRE1 axis of UPR can activate NF κ B, a key regulator in inflammation, by recruiting I κ B kinase (82). As a result, loss of IRE1 reduces the activation of NF κ B activation and production of TNF- α (82). In addition, the IRE1 axis can also activate JNK, and subsequently induce the expression of inflammatory genes by activating activator protein 1 (AP1) (83). ATF6, the third axis of UPR signaling, can also activate NF κ B pathway and induce inflammatory response. Therefore, suppression of ATF6 reduces NF κ B activation caused by BiP degradation (84).

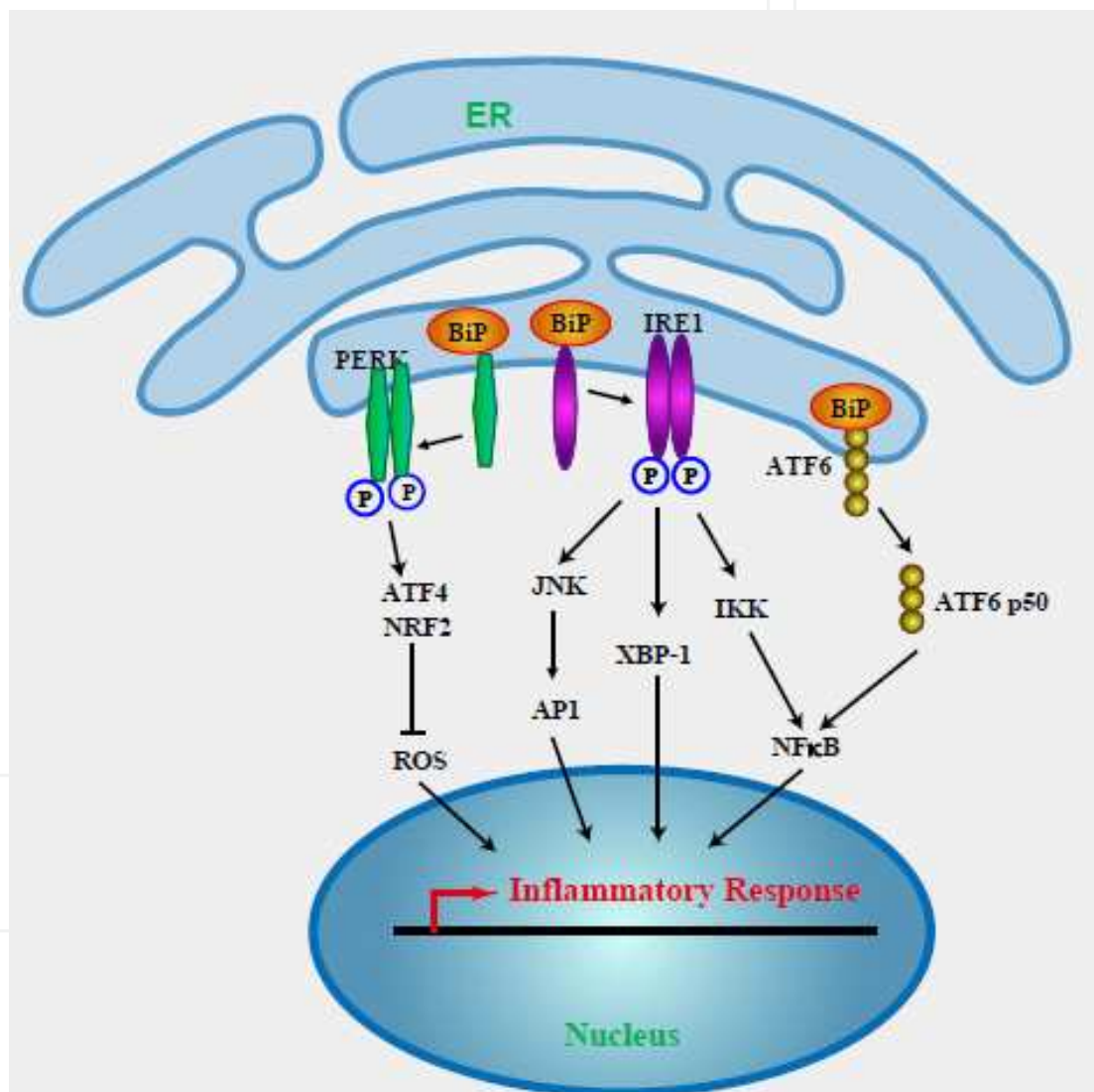


Figure 4. UPR-mediated inflammatory signaling. UPR regulates inflammation through a variety of mechanisms involving ROS, JNK, and NF κ B. PERK promotes ATF4 and NRF2, which then suppress ROS production by activating antioxidant pathway. Upon activation, IRE1/TRAF2 complex recruits IKK (I κ B Kinase), leading to the phosphorylation of I κ B α and subsequent activation of NF κ B. IRE1/TRAF2 can also activate JNK, followed by the activation of AP1. XBP-1 induced by IRE1 can also induce the expression of various genes implicated inflammation. Furthermore, cleaved ATF6 can promote inflammation via activating NF κ B.

ER stress regulates the expression of cytokines, while cytokines in turn may also induce ER stress via pathways including inducible nitric oxide synthase (iNOS) and JNK. JNK pathway is activated by IL-1 β . Suppression of JNK by its inhibitor SP600125 can protect β cells from IL-1 β -induced apoptosis (85). Inflammatory cytokines induce iNOS expression in β cells and produce copious amount of nitric oxygen (86). Nitric oxygen is an important mediator of β -cell death in type 1 diabetes. Excessive nitric oxygen can induce DNA damage, which leads to β cell apoptosis through p53 pathway or necrosis through poly (ADP-ribose) polymerase pathway (87). In addition, nitric oxygen can also deplete ER Ca²⁺ stores by activating Ca²⁺ channels or inhibiting Ca²⁺ pumps (88-90). Depletion of Ca²⁺ then leads to the activation of CHOP and induces ER stress and apoptosis of β cells (91;92).

4.4. ER stress in the autoimmune process of type 1 diabetes

Given the involvement of ER stress in both innate and adaptive immune systems, pathways of ER stress play a role in the autoimmune process of type 1 diabetes. For example, mice deficient in PERK, a molecule responsible for regulating UPR, are extremely susceptible to diabetes. Although the exocrine and endocrine pancreas developed normally, the *null* mice display a progressive loss of β mass and insulin insufficiency postnatally (93) (93). A severe defect of β cell proliferation and differentiation was also found in *PERK null* mice, resulting in low pancreatic β mass and proinsulin trafficking defects (94). Consistent with those observations in mice, some infant-onset diabetic cases in humans are confirmed to be associated with the mutations in PERK. For example, loss of *EIF2AK3* (the gene encodes PERK) develops Wolcott-Rallison syndrome, an autosomal recessive disorder featured by early infancy insulin-dependency and multiple systemic manifestations including growth retardation, hepatic/renal dysfunction, mental retardation, and cardiovascular abnormalities (86;95). Similarly, disruption of UPR by mutating eIF2 α , the downstream molecule of PERK signaling, enhances the sensitivity to ER stress-induced apoptosis and results in defective gluconeogenesis. Mice carrying a homozygous Ser51Ala mutation for eIF2 α show multiple defects in pancreatic β cells including the smaller core of insulin-secreting β cells and attenuated insulin secretion (41). Altogether, defects in PERK/eIF2 α signaling render β cells highly vulnerable to ER stress in both humans and mice (87;96). In addition to PERK/eIF2 α signaling, the other two pathways of ER stress, IRE1 and ATF6, are also implicated in the functionality of β cells. The activation of IRE1 signaling is involved in the insulin biosynthesis induced by hyperglycemia. Transient exposure to high glucose enhances IRE1 α phosphorylation without activation of XBP-1 and BiP dissociation. IRE1 α activation induced by transient exposure to high glucose induces insulin biosynthesis by up-regulating WFS1, a component involved in UPR and maintaining ER homeostasis (10;97). However, chronic exposure of β cells to high glucose may cause activation of IRE1 but with a different downstream signaling, leading to the suppression of insulin biosynthesis (10). The activation of ATF6 induced by ER stress also suppressed the expression of insulin by up-regulating orphan nuclear receptor small heterodimer partner (98).

5. The role of ER stress in β cell destruction

5.1. The involvement of ER stress in β cell destruction

Increasing evidence suggests an important role of ER stress in autoimmune-mediated β cell destruction (99;100). It was noted that β cell loss is the direct causing factor for insufficient insulin secretion in type 1 diabetes patients. Pancreatic β cells have a very well-developed ER to fulfill their biological function for secreting insulin and other glycoproteins, causing the high sensitivity of β cells to ER stress and the subsequent UPR. Severe or long-term ER stress would direct β cells undergoing apoptosis (99). As described earlier, all the three pathways of ER stress are important in the execution of β cell function and involved in the autoimmune responses during the process of type 1 diabetes.

Pro-inflammatory cytokines are believed as the major mediators contributing to ER stress in β cell mediated by autoimmune response. Autoreactive immune cells infiltrated in pancreas produce pro-inflammatory cytokines, the primary causing factor for β cell death in type 1 diabetes (101). Autoreactive macrophages and T-lymphocytes present in the pancreatic islets in the early stage of type 1 diabetes and secrete massive pro-inflammatory cytokines including IL-1 β , IFN- γ and TNF- α . Pro-inflammatory cytokines have been confirmed as strong inducers of ER stress in pancreatic β cells. Insult of β cells with IL-1 β and IFN- γ was reported to induce the expression of death protein 5, a protein involved in the cytokine-induced ER stress and β cell death (102). Suppression of death protein 5 by siRNA provides protection for β cells against pro-inflammatory cytokine-induced ER stress (102). In addition, stimulation of β cells with IL-1 β and IFN- γ can decrease the expression of sarcoendoplasmic reticulum pump Ca²⁺ ATPase 2b, leading to subsequent depletion of Ca²⁺ in the ER (103). It has been well demonstrated that altered ER Ca²⁺ concentration induces the accumulation of unfolded proteins in ER associated with the induction of UPR and ER stress in β cells (104). Reactive oxygen species such as nitric oxygen produced during inflammation are believed to play a critical role in ER stress-induced β cell death. Excessive nitric oxygen production during insulinitis induces β cell apoptosis in a CHOP-dependent manner (91).

In addition to cytokine-induced ER stress, defective protein processing and trafficking are also a direct cause of ER stress in β cell. For instance, mis-folding of insulin in β cells directly induces chronic ER stress as evidenced by the observations in Akita mice. The mutation of *Ins2* gene in Akita mouse disrupts a disulfide bond between α and β chain of proinsulin, leading to the mis-folding of the mutated insulin. This mutation therefore induces chronic ER stress in β cells and finally causes diabetes in Akita mouse (105). The inefficiency of protein trafficking from ER to Golgi apparatus also causes ER stress in β cells (106).

Hyperglycemia occurs only when β cells fail to compensate the increased demand for insulin. Therefore, β cells are usually "exhausted" in diabetic patients (87). The increased insulin demand requires the remaining functional β cells to increase insulin synthesis to compensate the decrease of β mass. The altered insulin synthesis causes ER stress in the β cells of patients with type 1 diabetes. In later case, this compensation is beneficial for control of blood glucose homeostasis in a short term. However, the long term alterations of insulin synthesis

in the β cells also induce ER stress which in turn exacerbates β cell dysfunction and promotes disease progression. Collectively, there is convincing evidence that ER stress plays an essential role in β cell destruction during the course of type 1 diabetes.

5.2. Mechanisms underlying ER stress-induced β cell death

The primary purpose of ER stress response is to compensate the damage caused by the disturbances of normal ER function. However, persistence of ER dysfunction would eventually render cells undergoing apoptosis. The mechanisms underlying ER stress induced cell death are not fully elucidated, due to the fact that multiple potential participants involved but little clarity on the dominant death effectors in a particular cellular context. Generally, the process of cell death by ER stress can be illustrated in three phases: adaptation, alarm, and apoptosis (39).

The adaptation response phase is to protect cells from damage induced by the disturbances of ER function and restore the homeostasis of ER. As described earlier, UPR signaling involves three axes of responses: IRE1, PERK, and ATF6. These axes interact between each other and form a feedback regulatory mechanism to control the activity of UPR. The accumulation of unfolded proteins in ER results in the engagement of ER resident chaperon BiP, and as a consequence, IRE1, PERK, and ATF6 are released from BiP. Therefore, over-expression of BiP can prevent cell death induced by oxidative stress, Ca^{2+} disturbances, and hypoxia (107). Upon ER stress, the transcription of BiP is enhanced by ATF6p50, the cleaved form of ATF6 (108). PERK is oligomerized and phosphorylated upon the release from BiP. Activated PERK inactivates eIF2 α to reduce mRNA translation and protein load on ER. Therefore, PERK deficiency results in an abnormally elevated protein synthesis in response to ER stress, and renders cells highly sensitive to ER stress-induced apoptosis (109). Consistently, as a downstream molecule of PERK, eIF2 α is required for cell survival upon the insult of ER stress. A mutation at the phosphorylation site of eIF2 α (Ser51Ala) abolishes the translational suppression in response to ER stress (41). When released from BiP, IRE1 becomes dimerized and activated. Activated IRE1 then induces XBP-1 by promoting the splicing of its mRNA (44). XBP-1 is responsible for the transcription of many adaptation genes implicated in UPR. Unlike PERK and IRE1, ATF6 translocates into the Golgi apparatus once released from BiP. The transmembrane domain of ATF6 is cleaved in the Golgi apparatus and is then relocated into the nucleus, by which it regulates gene expression (51).

During the alarm phase, many signal pathways are activated to alert the system. For instance, the cytoplasmic part of IRE1 can bind to TNF receptor-associated factor 2 (TRAF2), a key adaptor mediating TNF-induced innate immune response. TRAF2 then activates NF κ B pathway via activating IKK and activates the signaling for c-Jun N-terminal kinases (JNK) by apoptosis signal-regulating kinase 1 (Ask1). It is reported that dominant negative TRAF2 suppresses the activation of JNK in response to ER stress (110). In addition, TRAF2 is also a critical component for E3 ubiquitin-protein ligase complex (111). E3 ubiquitin-protein ligase complex binds to Ubc13 and mediates the noncanonical ubiquitination of substrates, which is suggested to be required for the activation of JNK (112).

Furthermore, IRE1 can also activate JNK signaling by interacting with c-Jun N-terminal inhibitory kinase (JIK) (113).

Although the purpose of UPR is to maintain the homeostasis of ER, apoptosis could occur when the insult of ER stress exceeds the cellular regulatory capacity. Apoptosis is initiated by the activation of several proteases including caspase-12, caspase-4, caspase-2, and caspase-9. Studies in rodents suggest that caspase-12 is activated by IRE1 and is involved in ER stress-induced apoptosis. Mice deficient for caspase-12 are resistant to ER stress-induced apoptosis, but remain susceptible to apoptosis induced by other stimuli (114). Caspase-12 can also be activated by TRAF2, a downstream molecule of IRE1 (113). In response to ER stress, caspase-7 is translocated from the cytosol to the ER surface, and then activates pro-caspase-12 (115). Human caspase-4, the closest paralog of rodent caspase-12, can only be activated by ER stress-inducing reagents not by the other apoptotic reagents. Knockdown of caspase-4 by siRNA reduces ER stress-induced apoptosis in neuroblastoma cells, suggesting the involvement of human caspase-4 in ER stress-induced cell death (116). Similarly, caspase-2 and caspase-9 are also activated in the early phase of ER stress. Inhibition of their activation either by inhibitors or siRNA reduces ER stress-induced apoptosis (117). Other than caspase proteins, Ask1 kinase and CHOP are also critical mediators for ER stress-induced cell death. IRE1/TRAF2 complex recruits Ask1 and activates subsequent JNK signaling. The activation of JNK then induces apoptosis by inhibiting anti-apoptotic protein BCL-2 (118) and inducing pro-apoptotic protein Bim (119;120). Deficiency of Ask1 suppresses ER stress-induced JNK activation and protects cells against ER stress-induced apoptosis (121). CHOP, a transcription factor belonging to basic leucine zipper transcription factor family, can be activated by many inducers of UPR including ATF4, ATF6, and XBP-1. Upon activation, CHOP induces cells undergoing apoptosis through suppressing anti-apoptotic protein BCL-2 (122-124).

6. Conclusions and future directions

Although exogenous insulin therapy partly compensates the function of β cells, it cannot regulate blood glucose as accurately as the action of endogenous insulin. As a result, long-term improperly control of blood glucose homeostasis predisposes patients with type 1 diabetes to the development of diverse complications such as diabetic retinopathy (125-127), nephropathy (128;129), neuropathy (130-132), foot ulcers (133-135), and cardiovascular diseases (136-138). Due to the long-term health consequences of diabetes, impact of insulin dependence on life quality, and increasing appearance in both young and old populations, understanding the pathophysiology of diabetes and finding a better way to treat diabetes has become a high priority. Although the underlying mechanisms leading to type 1 diabetes have yet to be fully addressed, accumulating evidence suggests that ER stress plays a critical role in autoimmune-mediated β cell destruction during the course of type 1 diabetes. ER stress in β cells can be triggered by either autoimmune responses against β -cell self-antigens or the increase of compensated insulin synthesis. During the course of type 1 diabetes, autoreactive immune cells secrete copious amount of inflamma-

tory cytokines, leading to excessive production of nitric oxide and β cell destruction in an ER stress-dependent pathway. ER stress also regulates the functionality of immune cells with implications in autoimmune progression. The inadequate insulin secretion in patients with type 1 diabetes renders the residual β cells for compensated insulin secretion to maintain blood glucose homeostasis. This increase in insulin biosynthesis could overwhelm the folding capacity of ER, and exacerbate β cell dysfunction by inducing ER stress in β cells.

Although ER stress is a critical factor involved in the pathogenesis of type 1 diabetes, it should be kept in mind that the mechanisms underlying autoimmune-mediated β cell destruction in type 1 diabetes are complex, and ER stress is unlikely the exclusive mechanism implicated in disease process. Despite recent significant progress in this area, there are still many questions yet to be addressed. Are there additional factors inducing ER stress in β cells during type 1 diabetes development? Can ER stress be served as a biomarker for β cell destruction and autoimmune progression in the clinic setting? Does blockade of ER stress in immune cells attenuate autoimmune progression and protect β cells? Future studies aimed to dissect these questions would provide a deep insight for type 1 diabetes pathogenesis and would have great potential for developing novel therapeutic strategies against this devastating disorder.

Acknowledgements

This work is supported by a grant from the National Natural Science Foundation of China (81101553/H1604) to JZ. The author declares no competing financial interest.

Abbreviations

AP1, activator protein 1; Ask1, apoptosis signal-regulating kinase 1; ATF6, Activating Transcription Factor 6; C/EBP, CCAAT/enhancer binding protein; CHOP, C/EBP homologous protein; CREBH, Cyclic-AMP-responsive-element-binding protein H; eIF2 α , eukaryotic initiation factor 2 α ; ER, Endoplasmic Reticulum; ER stress, Endoplasmic Reticulum stress; iNOS, inducible nitric oxide synthase; IRE1, inositol-requiring enzyme 1; IRS-1, insulin receptor substrate-1; JIK, c-Jun N-terminal inhibitory kinase; JNK, c-Jun N-terminal kinases; NRF2, nuclear factor-erythroid-derived 2-related factor 2; PERK, pancreatic endoplasmic reticulum kinase; RER, rough endoplasmic reticulum; ROS, reactive oxygen species; SER, smooth endoplasmic reticulum; TLR, Toll-like receptor; TRAF2, TNF receptor-associated factor 2; TRIF, Toll-IL-1R-containing adaptor inducing IFN- β ; UPR, unfolded protein response; XBP-1, X box protein-1.

Author details

Jixin Zhong^{1,2}

Address all correspondence to: zhongjixin620@163.com

1 Department of Medicine, Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong, China

2 Davis Heart & Lung Research Institute, The Ohio State University College of Medicine, Columbus, Ohio, USA

References

- [1] Espino-Paisan L, Urcelay E, Concha EGdl, Santiago JL: Early and Late Onset Type 1 Diabetes: One and the Same or Two Distinct Genetic Entities? In Type 1 Diabetes Complications. Wagner D, Ed. InTech, 2011,
- [2] Maahs,DM, West,NA, Lawrence,JM, Mayer-Davis,EJ: Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am* 39:481-497, 2010
- [3] Lehuen,A, Diana,J, Zacccone,P, Cooke,A: Immune cell crosstalk in type 1 diabetes. *Nat Rev Immunol* 10:501-513, 2010
- [4] Zhong J, Xu J, Yang P, Liang Y, Wang C-Y: Innate immunity in the recognition of beta-cell antigens in type 1 diabetes. In Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy. Wagner D, Ed. InTech, 2011,
- [5] American Diabetes Association: Standards of medical care in diabetes--2006. *Diabetes Care* 29 Suppl 1:S4-42, 2006
- [6] Tortora GJ, Derrickson BH: Principles of Anatomy and Physiology. John Wiley & Sons Inc., 2008,
- [7] Layden,VT, Durai,V, Lowe,WL: G-Protein-Coupled Receptors, Pancreatic Islets, and Diabetes. *Nature Education* 3:13, 2010
- [8] Gerich,JE: The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 19:491-503, 1998
- [9] Lillioja,S, Mott,DM, Spraul,M, Ferraro,R, Foley,JE, Ravussin,E, Knowler,WC, Bennett,PH, Bogardus,C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 329:1988-1992, 1993

- [10] Lipson,KL, Fonseca,SG, Ishigaki,S, Nguyen,LX, Foss,E, Bortell,R, Rossini,AA, Ura-no,F: Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab* 4:245-254, 2006
- [11] Rhodes CJ: Processing of the insulin molecule. in: D. LeRoith, S.I. Taylor, J.M. Olefsky (Eds.), *Diabetes Mellitus*, Lippincott Williams & Wilkins. In *Diabetes Mellitus: A Fundamental and Clinical Text*. 3rd ed. LeRoith D, Taylor SI, Olefsky JM, Eds. Lippincott Williams & Wilkins, 2003,
- [12] D'Hertog,W, Maris,M, Ferreira,GB, Verdrengh,E, Lage,K, Hansen,DA, Cardozo,AK, Workman,CT, Moreau,Y, Eizirik,DL, Waelkens,E, Overbergh,L, Mathieu,C: Novel insights into the global proteome responses of insulin-producing INS-1E cells to different degrees of endoplasmic reticulum stress. *J Proteome Res* 9:5142-5152, 2010
- [13] Hubbard,SC, Ivatt,RJ: Synthesis and processing of asparagine-linked oligosaccharides. *Annu Rev Biochem* 50:555-583, 1981
- [14] Kornfeld,R, Kornfeld,S: Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* 54:631-664, 1985
- [15] Fewell,SW, Travers,KJ, Weissman,JS, Brodsky,JL: The action of molecular chaperones in the early secretory pathway. *Annu Rev Genet* 35:149-191, 2001
- [16] Paltauf F, Kohlwein S.D., Henry S.A.: Regulation and compartmentalization of lipid synthesis in yeast. In *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Jones E.W., Broach J.R., Eds. NY, Cold Spring Harbor Laboratory Press, 1992, p. 415-500
- [17] Stevens,FJ, Argon,Y: Protein folding in the ER. *Semin Cell Dev Biol* 10:443-454, 1999
- [18] Schroder,M: Endoplasmic reticulum stress responses. *Cell Mol Life Sci* 65:862-894, 2008
- [19] Suzuki,CK, Bonifacino,JS, Lin,AY, Davis,MM, Klausner,RD: Regulating the retention of T-cell receptor alpha chain variants within the endoplasmic reticulum: Ca(2+)-dependent association with BiP. *J Cell Biol* 114:189-205, 1991
- [20] Li,LJ, Li,X, Ferrario,A, Rucker,N, Liu,ES, Wong,S, Gomer,CJ, Lee,AS: Establishment of a Chinese hamster ovary cell line that expresses grp78 antisense transcripts and suppresses A23187 induction of both GRP78 and GRP94. *J Cell Physiol* 153:575-582, 1992
- [21] Corbett,EF, Oikawa,K, Francois,P, Tessier,DC, Kay,C, Bergeron,JJ, Thomas,DY, Krause,KH, Michalak,M: Ca²⁺ regulation of interactions between endoplasmic reticulum chaperones. *J Biol Chem* 274:6203-6211, 1999
- [22] Zhang,JX, Braakman,I, Matlack,KE, Helenius,A: Quality control in the secretory pathway: the role of calreticulin, calnexin and BiP in the retention of glycoproteins with C-terminal truncations. *Mol Biol Cell* 8:1943-1954, 1997

- [23] Orrenius,S, Zhivotovsky,B, Nicotera,P: Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 4:552-565, 2003
- [24] Lee,AS: The glucose-regulated proteins: stress induction and clinical applications. *Trends Biochem Sci* 26:504-510, 2001
- [25] Schroder,M, Friedl,P: Overexpression of recombinant human antithrombin III in Chinese hamster ovary cells results in malformation and decreased secretion of recombinant protein. *Biotechnol Bioeng* 53:547-559, 1997
- [26] Schroder,M, Schafer,R, Friedl,P: Induction of protein aggregation in an early secretory compartment by elevation of expression level. *Biotechnol Bioeng* 78:131-140, 2002
- [27] Dorner,AJ, Wasley,LC, Kaufman,RJ: Increased synthesis of secreted proteins induces expression of glucose-regulated proteins in butyrate-treated Chinese hamster ovary cells. *J Biol Chem* 264:20602-20607, 1989
- [28] Kaufman,RJ, Wasley,LC, Dorner,AJ: Synthesis, processing, and secretion of recombinant human factor VIII expressed in mammalian cells. *J Biol Chem* 263:6352-6362, 1988
- [29] Schroder,M, Kaufman,RJ: ER stress and the unfolded protein response. *Mutat Res* 569:29-63, 2005
- [30] Reimold,AM, Iwakoshi,NN, Manis,J, Vallabhajosyula,P, Szomolanyi-Tsuda,E, Grallese,EM, Friend,D, Grusby,MJ, Alt,F, Glimcher,LH: Plasma cell differentiation requires the transcription factor XBP-1. *Nature* 412:300-307, 2001
- [31] Iwakoshi,NN, Lee,AH, Vallabhajosyula,P, Otipoby,KL, Rajewsky,K, Glimcher,LH: Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nat Immunol* 4:321-329, 2003
- [32] Martinez,IM, Chrispeels,MJ: Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes. *Plant Cell* 15:561-576, 2003
- [33] Pakula,TM, Laxell,M, Huuskonen,A, Uusitalo,J, Saloheimo,M, Penttila,M: The effects of drugs inhibiting protein secretion in the filamentous fungus *Trichoderma reesei*. Evidence for down-regulation of genes that encode secreted proteins in the stressed cells. *J Biol Chem* 278:45011-45020, 2003
- [34] Harding,HP, Zhang,Y, Ron,D: Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397:271-274, 1999
- [35] Casagrande,R, Stern,P, Diehn,M, Shamu,C, Osario,M, Zuniga,M, Brown,PO, Ploegh,H: Degradation of proteins from the ER of *S. cerevisiae* requires an intact unfolded protein response pathway. *Mol Cell* 5:729-735, 2000
- [36] Friedlander,R, Jarosch,E, Urban,J, Volkwein,C, Sommer,T: A regulatory link between ER-associated protein degradation and the unfolded-protein response. *Nat Cell Biol* 2:379-384, 2000

- [37] Travers,KJ, Patil,CK, Wodicka,L, Lockhart,DJ, Weissman,JS, Walter,P: Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101:249-258, 2000
- [38] Kaufman,RJ: Orchestrating the unfolded protein response in health and disease. *J Clin Invest* 110:1389-1398, 2002
- [39] Xu,C, Bailly-Maitre,B, Reed,JC: Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 115:2656-2664, 2005
- [40] Kaufman,RJ, Scheuner,D, Schroder,M, Shen,X, Lee,K, Liu,CY, Arnold,SM: The unfolded protein response in nutrient sensing and differentiation. *Nat Rev Mol Cell Biol* 3:411-421, 2002
- [41] Scheuner,D, Song,B, McEwen,E, Liu,C, Laybutt,R, Gillespie,P, Saunders,T, Bonner-Weir,S, Kaufman,RJ: Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell* 7:1165-1176, 2001
- [42] Tirasophon,W, Welihinda,AA, Kaufman,RJ: A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev* 12:1812-1824, 1998
- [43] Wang,XZ, Harding,HP, Zhang,Y, Jolicoeur,EM, Kuroda,M, Ron,D: Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* 17:5708-5717, 1998
- [44] Lee,K, Tirasophon,W, Shen,X, Michalak,M, Prywes,R, Okada,T, Yoshida,H, Mori,K, Kaufman,RJ: IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* 16:452-466, 2002
- [45] Yoshida,H, Matsui,T, Yamamoto,A, Okada,T, Mori,K: XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107:881-891, 2001
- [46] Calton,M, Zeng,H, Urano,F, Till,JH, Hubbard,SR, Harding,HP, Clark,SG, Ron,D: IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415:92-96, 2002
- [47] Lee,AH, Iwakoshi,NN, Glimcher,LH: XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 23:7448-7459, 2003
- [48] Yoshida,H, Matsui,T, Hosokawa,N, Kaufman,RJ, Nagata,K, Mori,K: A time-dependent phase shift in the mammalian unfolded protein response. *Dev Cell* 4:265-271, 2003
- [49] Lee,AH, Chu,GC, Iwakoshi,NN, Glimcher,LH: XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J* 24:4368-4380, 2005
- [50] Hollien,J, Weissman,JS: Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* 313:104-107, 2006

- [51] Ye,J, Rawson,RB, Komuro,R, Chen,X, Dave,UP, Prywes,R, Brown,MS, Goldstein,JL: ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 6:1355-1364, 2000
- [52] Okada,T, Yoshida,H, Akazawa,R, Negishi,M, Mori,K: Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. *Biochem J* 366:585-594, 2002
- [53] Ni,M, Lee,AS: ER chaperones in mammalian development and human diseases. *FEBS Lett* 581:3641-3651, 2007
- [54] Han,J, Zhong,J, Wei,W, Wang,Y, Huang,Y, Yang,P, Purohit,S, Dong,Z, Wang,MH, She,JX, Gong,F, Stern,DM, Wang,CY: Extracellular high-mobility group box 1 acts as an innate immune mediator to enhance autoimmune progression and diabetes onset in NOD mice. *Diabetes* 57:2118-2127, 2008
- [55] Zhang,S, Zhong,J, Yang,P, Gong,F, Wang,CY: HMGB1, an innate alarmin, in the pathogenesis of type 1 diabetes. *Int J Clin Exp Pathol* 3:24-38, 2009
- [56] Zhong J, Yang P, Wang C: Environmental Triggers and Endogenous Alarmins Linking Innate Immunity to the Pathogenesis of Type 1 Diabetes. In: *Type 1 Diabetes Mellitus: Etiology, Diagnosis and Treatment*. Nova Science Publishers, Inc., 2011, p. 177-206
- [57] Zhong J, Xu J, Yang P, Liang Y, Wang C-Y: Innate immunity in the recognition of ? cell antigens in type 1 diabetes. In *Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy*. Wagner D, Ed. InTech, 2011,
- [58] Pietropaolo,M, Barinas-Mitchell,E, Kuller,LH: The heterogeneity of diabetes: unraveling a dispute: is systemic inflammation related to islet autoimmunity? *Diabetes* 56:1189-1197, 2007
- [59] Zhao,L, Ackerman,SL: Endoplasmic reticulum stress in health and disease. *Curr Opin Cell Biol* 18:444-452, 2006
- [60] Zhang,K, Shen,X, Wu,J, Sakaki,K, Saunders,T, Rutkowski,DT, Back,SH, Kaufman,RJ: Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124:587-599, 2006
- [61] Iwakoshi,NN, Pypaert,M, Glimcher,LH: The transcription factor XBP-1 is essential for the development and survival of dendritic cells. *J Exp Med* 204:2267-2275, 2007
- [62] Goodall,JC, Wu,C, Zhang,Y, McNeill,L, Ellis,L, Saudek,V, Gaston,JS: Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc Natl Acad Sci U S A* 107:17698-17703, 2010
- [63] Richardson,CE, Kooistra,T, Kim,DH: An essential role for XBP-1 in host protection against immune activation in *C. elegans*. *Nature* 463:1092-1095, 2010

- [64] Kaser,A, Lee,AH, Franke,A, Glickman,JN, Zeissig,S, Tilg,H, Nieuwenhuis,EE, Higgins,DE, Schreiber,S, Glimcher,LH, Blumberg,RS: XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 134:743-756, 2008
- [65] Woo,CW, Cui,D, Arellano,J, Dorweiler,B, Harding,H, Fitzgerald,KA, Ron,D, Tabas,I: Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by toll-like receptor signalling. *Nat Cell Biol* 11:1473-1480, 2009
- [66] Baekkeskov,S, Nielsen,JH, Marnier,B, Bilde,T, Ludvigsson,J, Lernmark,A: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 298:167-169, 1982
- [67] Gass,JN, Gifford,NM, Brewer,JW: Activation of an unfolded protein response during differentiation of antibody-secreting B cells. *J Biol Chem* 277:49047-49054, 2002
- [68] Zhang,K, Wong,HN, Song,B, Miller,CN, Scheuner,D, Kaufman,RJ: The unfolded protein response sensor IRE1alpha is required at 2 distinct steps in B cell lymphopoiesis. *J Clin Invest* 115:268-281, 2005
- [69] Niu,H, Ye,BH, la-Favera,R: Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev* 12:1953-1961, 1998
- [70] Lin,KI, ngelin-Duclos,C, Kuo,TC, Calame,K: Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. *Mol Cell Biol* 22:4771-4780, 2002
- [71] Reimold,AM, Ponath,PD, Li,YS, Hardy,RR, David,CS, Strominger,JL, Glimcher,LH: Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. *J Exp Med* 183:393-401, 1996
- [72] Shaffer,AL, Shapiro-Shelef,M, Iwakoshi,NN, Lee,AH, Qian,SB, Zhao,H, Yu,X, Yang,L, Tan,BK, Rosenwald,A, Hurt,EM, Petroulakis,E, Sonenberg,N, Yewdell,JW, Calame,K, Glimcher,LH, Staudt,LM: XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* 21:81-93, 2004
- [73] Gass,JN, Jiang,HY, Wek,RC, Brewer,JW: The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells. *Mol Immunol* 45:1035-1043, 2008
- [74] Pino,SC, O'Sullivan-Murphy,B, Lidstone,EA, Thornley,TB, Jurczyk,A, Urano,F, Greiner,DL, Mordes,JP, Rossini,AA, Bortell,R: Protein kinase C signaling during T cell activation induces the endoplasmic reticulum stress response. *Cell Stress Chaperones* 13:421-434, 2008
- [75] Kamimura,D, Bevan,MJ: Endoplasmic reticulum stress regulator XBP-1 contributes to effector CD8+ T cell differentiation during acute infection. *J Immunol* 181:5433-5441, 2008

- [76] Pino,SC, O'Sullivan-Murphy,B, Lidstone,EA, Yang,C, Lipson,KL, Jurczyk,A, diIorio,P, Brehm,MA, Mordes,JP, Greiner,DL, Rossini,AA, Bortell,R: CHOP mediates endoplasmic reticulum stress-induced apoptosis in Gimap5-deficient T cells. *PLoS One* 4:e5468, 2009
- [77] Malhotra,JD, Kaufman,RJ: Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal* 9:2277-2293, 2007
- [78] Tu,BP, Weissman,JS: Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* 164:341-346, 2004
- [79] Harding,HP, Zhang,Y, Zeng,H, Novoa,I, Lu,PD, Calton,M, Sadri,N, Yun,C, Popko,B, Paules,R, Stojdl,DF, Bell,JC, Hettmann,T, Leiden,JM, Ron,D: An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 11:619-633, 2003
- [80] Cullinan,SB, Zhang,D, Hannink,M, Arvisais,E, Kaufman,RJ, Diehl,JA: Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* 23:7198-7209, 2003
- [81] Cullinan,SB, Diehl,JA: PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 279:20108-20117, 2004
- [82] Hu,P, Han,Z, Couvillon,AD, Kaufman,RJ, Exton,JH: Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* 26:3071-3084, 2006
- [83] Davis,RJ: Signal transduction by the JNK group of MAP kinases. *Cell* 103:239-252, 2000
- [84] Yamazaki,H, Hiramatsu,N, Hayakawa,K, Tagawa,Y, Okamura,M, Ogata,R, Huang,T, Nakajima,S, Yao,J, Paton,AW, Paton,JC, Kitamura,M: Activation of the Akt-NF-kappaB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. *J Immunol* 183:1480-1487, 2009
- [85] Wang,Q, Zhang,H, Zhao,B, Fei,H: IL-1beta caused pancreatic beta-cells apoptosis is mediated in part by endoplasmic reticulum stress via the induction of endoplasmic reticulum Ca²⁺ release through the c-Jun N-terminal kinase pathway. *Mol Cell Biochem* 324:183-190, 2009
- [86] Delepine,M, Nicolino,M, Barrett,T, Golamaully,M, Lathrop,GM, Julier,C: EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. *Nat Genet* 25:406-409, 2000
- [87] Oyadomari,S, Mori,M: Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death Differ* 11:381-389, 2004

- [88] Messmer,UK, Brune,B: Nitric oxide-induced apoptosis: p53-dependent and p53-independent signalling pathways. *Biochem J* 319 (Pt 1):299-305, 1996
- [89] Viner,RI, Ferrington,DA, Williams,TD, Bigelow,DJ, Schoneich,C: Protein modification during biological aging: selective tyrosine nitration of the SERCA2a isoform of the sarcoplasmic reticulum Ca²⁺-ATPase in skeletal muscle. *Biochem J* 340 (Pt 3): 657-669, 1999
- [90] Xu,KY, Huso,DL, Dawson,TM, Bredt,DS, Becker,LC: Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci U S A* 96:657-662, 1999
- [91] Oyadomari,S, Takeda,K, Takiguchi,M, Gotoh,T, Matsumoto,M, Wada,I, Akira,S, Araki,E, Mori,M: Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. *Proc Natl Acad Sci U S A* 98:10845-10850, 2001
- [92] Xu,L, Eu,JP, Meissner,G, Stamler,JS: Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 279:234-237, 1998
- [93] Harding,HP, Zeng,H, Zhang,Y, Jungries,R, Chung,P, Plesken,H, Sabatini,DD, Ron,D: Diabetes mellitus and exocrine pancreatic dysfunction in perk^{-/-} mice reveals a role for translational control in secretory cell survival. *Mol Cell* 7:1153-1163, 2001
- [94] Zhang,W, Feng,D, Li,Y, Iida,K, McGrath,B, Cavener,DR: PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab* 4:491-497, 2006
- [95] Araki,E, Oyadomari,S, Mori,M: Endoplasmic reticulum stress and diabetes mellitus. *Intern Med* 42:7-14, 2003
- [96] Mathis,D, Vence,L, Benoist,C: beta-Cell death during progression to diabetes. *Nature* 414:792-798, 2001
- [97] Fonseca,SG, Fukuma,M, Lipson,KL, Nguyen,LX, Allen,JR, Oka,Y, Urano,F: WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. *J Biol Chem* 280:39609-39615, 2005
- [98] Seo,HY, Kim,YD, Lee,KM, Min,AK, Kim,MK, Kim,HS, Won,KC, Park,JY, Lee,KU, Choi,HS, Park,KG, Lee,IK: Endoplasmic reticulum stress-induced activation of activating transcription factor 6 decreases insulin gene expression via up-regulation of orphan nuclear receptor small heterodimer partner. *Endocrinology* 149:3832-3841, 2008
- [99] Eizirik,DL, Cardozo,AK, Cnop,M: The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev* 29:42-61, 2008
- [100] Laybutt,DR, Preston,AM, Akerfeldt,MC, Kench,JG, Busch,AK, Biankin,AV, Biden,TJ: Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* 50:752-763, 2007

- [101] Eizirik,DL, Colli,ML, Ortis,F: The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. *Nat Rev Endocrinol* 5:219-226, 2009
- [102] Gurzov,EN, Ortis,F, Cunha,DA, Gosset,G, Li,M, Cardozo,AK, Eizirik,DL: Signaling by IL-1 β +IFN- γ and ER stress converge on DP5/Hrk activation: a novel mechanism for pancreatic beta-cell apoptosis. *Cell Death Differ* 16:1539-1550, 2009
- [103] Cardozo,AK, Ortis,F, Storling,J, Feng,YM, Rasschaert,J, Tonnesen,M, Van,EF, Mandrup-Poulsen,T, Herchuelz,A, Eizirik,DL: Cytokines downregulate the sarcoendoplasmic reticulum pump Ca²⁺ ATPase 2b and deplete endoplasmic reticulum Ca²⁺, leading to induction of endoplasmic reticulum stress in pancreatic beta-cells. *Diabetes* 54:452-461, 2005
- [104] Rutkowski,DT, Kaufman,RJ: A trip to the ER: coping with stress. *Trends Cell Biol* 14:20-28, 2004
- [105] Ron,D: Proteotoxicity in the endoplasmic reticulum: lessons from the Akita diabetic mouse. *J Clin Invest* 109:443-445, 2002
- [106] Preston,AM, Gurisik,E, Bartley,C, Laybutt,DR, Biden,TJ: Reduced endoplasmic reticulum (ER)-to-Golgi protein trafficking contributes to ER stress in lipotoxic mouse beta cells by promoting protein overload. *Diabetologia* 52:2369-2373, 2009
- [107] Liu,H, Bowes,RC, III, van de,WB, Sillence,C, Nagelkerke,JF, Stevens,JL: Endoplasmic reticulum chaperones GRP78 and calreticulin prevent oxidative stress, Ca²⁺ disturbances, and cell death in renal epithelial cells. *J Biol Chem* 272:21751-21759, 1997
- [108] Haeri,M, Knox,BE: Endoplasmic Reticulum Stress and Unfolded Protein Response Pathways: Potential for Treating Age-related Retinal Degeneration. *J Ophthalmic Vis Res* 7:45-59, 2012
- [109] Harding,HP, Zhang,Y, Bertolotti,A, Zeng,H, Ron,D: Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* 5:897-904, 2000
- [110] Urano,F, Wang,X, Bertolotti,A, Zhang,Y, Chung,P, Harding,HP, Ron,D: Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287:664-666, 2000
- [111] Zheng,C, Kabaleeswaran,V, Wang,Y, Cheng,G, Wu,H: Crystal structures of the TRAF2: cIAP2 and the TRAF1: TRAF2: cIAP2 complexes: affinity, specificity, and regulation. *Mol Cell* 38:101-113, 2010
- [112] Habelhah,H, Takahashi,S, Cho,SG, Kadoya,T, Watanabe,T, Ronai,Z: Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF- κ B. *EMBO J* 23:322-332, 2004
- [113] Yoneda,T, Imaizumi,K, Oono,K, Yui,D, Gomi,F, Katayama,T, Tohyama,M: Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor ne-

crosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem* 276:13935-13940, 2001

- [114] Nakagawa,T, Zhu,H, Morishima,N, Li,E, Xu,J, Yankner,BA, Yuan,J: Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* 403:98-103, 2000
- [115] Rao,RV, Hermel,E, Castro-Obregon,S, del,RG, Ellerby,LM, Ellerby,HM, Bredesen,DE: Coupling endoplasmic reticulum stress to the cell death program. Mechanism of caspase activation. *J Biol Chem* 276:33869-33874, 2001
- [116] Hitomi,J, Katayama,T, Eguchi,Y, Kudo,T, Taniguchi,M, Koyama,Y, Manabe,T, Yamagishi,S, Bando,Y, Imaizumi,K, Tsujimoto,Y, Tohyama,M: Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death. *J Cell Biol* 165:347-356, 2004
- [117] Cheung,HH, Lynn,KN, Liston,P, Korneluk,RG: Involvement of caspase-2 and caspase-9 in endoplasmic reticulum stress-induced apoptosis: a role for the IAPs. *Exp Cell Res* 312:2347-2357, 2006
- [118] Yamamoto,K, Ichijo,H, Korsmeyer,SJ: BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. *Mol Cell Biol* 19:8469-8478, 1999
- [119] Putcha,GV, Le,S, Frank,S, Besirli,CG, Clark,K, Chu,B, Alix,S, Youle,RJ, LaMarche,A, Maroney,AC, Johnson,EM, Jr.: JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron* 38:899-914, 2003
- [120] Lei,K, Davis,RJ: JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci U S A* 100:2432-2437, 2003
- [121] Nishitoh,H, Matsuzawa,A, Tobiume,K, Saegusa,K, Takeda,K, Inoue,K, Hori,S, Kikuzuka,A, Ichijo,H: ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* 16:1345-1355, 2002
- [122] McCullough,KD, Martindale,JL, Klotz,LO, Aw,TY, Holbrook,NJ: Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol Cell Biol* 21:1249-1259, 2001
- [123] Matsumoto,M, Minami,M, Takeda,K, Sakao,Y, Akira,S: Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastic leukemia cells. *FEBS Lett* 395:143-147, 1996
- [124] Zinszner,H, Kuroda,M, Wang,X, Batchvarova,N, Lightfoot,RT, Remotti,H, Stevens,JL, Ron,D: CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev* 12:982-995, 1998
- [125] Bandurska-Stankiewicz,E, Wiatr,D: [Programme preventing vision loss due to diabetes]. *Klin Oczna* 109:359-362, 2007

- [126] Cheung,N, Wong,TY: Diabetic retinopathy and systemic vascular complications. *Prog Retin Eye Res* 27:161-176, 2008
- [127] Studholme,S: Diabetic retinopathy. *J Perioper Pract* 18:205-210, 2008
- [128] Monhart,V: [Diabetes mellitus, hypertension and kidney]. *Vnitr Lek* 54:499-504, 507, 2008
- [129] Navarro-Gonzalez,JF, Mora-Fernandez,C: The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* 19:433-442, 2008
- [130] Boulton,AJ: Diabetic neuropathy: classification, measurement and treatment. *Curr Opin Endocrinol Diabetes Obes* 14:141-145, 2007
- [131] Cornell,RS, Ducic,I: Painful diabetic neuropathy. *Clin Podiatr Med Surg* 25:347-360, 2008
- [132] Otto-Buczkowska,E, Kazibutowska,Z, Soltyk,J, Machnica,L: [Neuropathy and type 1 diabetes mellitus]. *Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw* 14:109-116, 2008
- [133] Gardner,SE, Frantz,RA: Wound bioburden and infection-related complications in diabetic foot ulcers. *Biol Res Nurs* 10:44-53, 2008
- [134] Malgrange,D: [Physiopathology of the diabetic foot]. *Rev Med Interne* 29 Suppl 2:S231-S237, 2008
- [135] Ochoa,O, Torres,FM, Shireman,PK: Chemokines and diabetic wound healing. *Vascular* 15:350-355, 2007
- [136] Anselmino,M, Gohlke,H, Mellbin,L, Ryden,L: Cardiovascular prevention in patients with diabetes and prediabetes. *Herz* 33:170-177, 2008
- [137] Inoguchi,T, Takayanagi,R: [Role of oxidative stress in diabetic vascular complications]. *Fukuoka Igaku Zasshi* 99:47-55, 2008
- [138] Marwick,TH: Diabetic heart disease. *Postgrad Med J* 84:188-192, 2008

