

ER stress and development of type 1 diabetes

Feyza Engin

Departments of Biomolecular Chemistry and Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

Correspondence to

Feyza Engin, Departments of Biomolecular Chemistry and Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Wisconsin-Madison School of Medicine and Public Health, 440 Henry Mall, Madison, WI, USA; fengin@wisc.edu

Received 13 April 2015

Revised 25 June 2015

Accepted 1 July 2015

ABSTRACT

Type 1 diabetes (T1D) results from an autoimmune-mediated destruction of pancreatic β cells. The incidence of T1D is on the rise globally around 3% to 5% per year and rapidly increasing incidence in younger children is of the greatest concern. currently, there is no way to cure or prevent T1D; hence, a deeper understanding of the underlying molecular mechanisms of this disease is essential to the development of new effective therapies. The endoplasmic reticulum (ER) is an organelle with multiple functions that are essential for cellular homeostasis. Excessive demand on the ER, chronic inflammation, and environmental factors lead to ER stress and to re-establish cellular homeostasis, the adaptive unfolded protein response (UPR) is triggered. However, chronic ER stress leads to a switch from a prosurvival to a proapoptotic UPR, resulting in cell death. Accumulating data have implicated ER stress and defective UPR in the pathogenesis of inflammatory and autoimmune diseases, and ER stress has been implicated in β -cell failure in type 2 diabetes. However, the role of ER stress and the UPR in β -cell pathophysiology and in the initiation and propagation of the autoimmune responses in T1D remains undefined. This review will highlight the current understanding and recent *in vivo* data on the role of ER stress and adaptive responses in T1D pathogenesis and the potential therapeutic aspect of enhancing β -cell ER function and restoring UPR defects as novel clinical strategies against this disease.

Type 1 diabetes (T1D) results from the immune-mediated destruction of pancreatic β cells leading to loss of insulin production, unsuppressed glucose production, and hyperglycemia.^{1–3} The prevalence of T1D between the ages 0 and 19 years was reported to be 1.7/1000 and has been rising globally and by as much as a 5.3% annually in the United States.⁴ Despite intensive research in the T1D field, the initial signals that trigger autoimmunity, the intracellular mediators that lead to destruction of β cells, and the crosstalk between β cells and immune cells in T1D still remain poorly understood, which hampers the development of effective preventive and/or therapeutic strategies against this disease.

Autoimmune T1D and metabolic disorder type 2 diabetes (T2D) have long been thought to show clear differences in disease prevalence, onset, weight, presence of autoantibodies, and insulinitis. However, emerging data suggest that T1D and T2D may show many more

similarities in disease pathogenesis than previously thought.^{5, 6} It is now becoming more evident that T2D patients also present with inflammation, earlier disease onset, and islet autoimmunity.^{7–11} In contrast, increased childhood obesity is seen more often in T1D patients¹² and was once called juvenile diabetes. Type 1 diabetes can be detected in adult patients as well. This convergence or the overlap between autoimmune T1D and metabolic disorder T2D suggests the possibility of common cellular and/or immunometabolic mechanisms.

In T1D, autoimmune responses lead to production of cytokines that cause an inflammatory state in β cells. In T2D, obesity-induced chronic low-grade systemic inflammation is observed in metabolic organs such as the liver, skeletal muscle, adipose tissue, hypothalamus, and β cells. Because accumulating data suggest a significant role for inflammation in diabetes pathogenesis, conditions that induce inflammation and/or disrupt cellular homeostasis have been critically important for understanding the molecular mechanism of this disease. One such condition has been proposed to be the stress in the endoplasmic reticulum (ER). Although a growing body of work linked ER stress to T2D and obesity pathogenesis both in mouse models and in human patients,^{13–15} the role of ER stress and cellular adaptive responses in T1D pathogenesis still remains unclear.

ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE

Endoplasmic reticulum stress and the adaptive unfolded protein response (UPR) have been of great interest lately due to their crucial role in the pathogenesis of multiple diseases including neurodegenerative diseases, cancer, and metabolic disorders. Surplus nutrition, viruses, environmental toxins, and chronic inflammation can alter calcium levels, protein glycosylation, and redox status in the ER leading to abnormal protein folding and secretion. Accumulation of unfolded and misfolded proteins results in ER stress, and the cells react to this stress conditions by activating an evolutionarily conserved adaptive response, the UPR.¹⁶ The UPR responds to ER stress primarily by arresting protein translation and activating signaling pathways that lead to expression of molecular chaperones that assist protein folding or facilitate degradation of misfolded proteins. To date, 3 major branches of the UPR have been identified. Ribonucleic acid-dependent



To cite: Engin F.
J Investig Med
2016;**64**:2–6.

protein kinase-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1) are all located in the ER membrane and are activated in response to the accumulation of unfolded proteins within the ER. Activation of PERK leads to phosphorylation of eukaryotic translation initiation factor 2α , which causes general inhibition of protein synthesis. In response to ER stress, ATF6 is translocated to the Golgi apparatus where it is cleaved by site 1 and site 2 proteases, yielding an active transcription factor. Similarly, activated IRE1 catalyzes removal of a small intron from the mRNA of the gene encoding X-box binding protein 1 (XBP1). This splicing event creates a translational frame shift in *XBP1* mRNA to produce an active transcription factor (sXBP1). Active ATF6 and sXBP1 subsequently bind to the ER stress response element and the UPR element of target genes to regulate their expression (figure 1). On acute stress, these branches of the UPR engage prosurvival and adaptive signaling, leading to re-establishment of cellular homeostasis; however, if ER stress is prolonged and unresolved, the UPR switches from a proadaptive to proapoptotic outcome. Thus, the UPR determines the homeostatic versus apo- ptotic fate of a cell.^{17 18}

INFLAMMATION, AUTOIMMUNITY, AND THE UPR

It has been demonstrated that chronic ER stress can engage the canonical branches of the UPR to elevate inflammation through activation of the c-Jun N-terminal kinase and I κ B kinase pathways. Reciprocally, inflammatory cytokines such as IL1- β , TNF- α , and IFN- γ were shown to induce ER stress through nitric oxide (NO).¹⁹ Production of NO leads to the depletion of ER calcium and inhibition of ER chaperone function resulting in a severe disruption in ER homeostasis.^{20 21} In addition, recent data support that UPR mediators can activate the nod-like receptor family, pryin domain containing-3 inflammasome and promote programmed cell death under chronic ER stress conditions.²²⁻²⁴ Thus, these

data indicate a tight interplay between inflammation and ER stress and point toward a possible connection with the immune response. Consistent with this notion, accumulating experimental evidence indicates that a dysregulated UPR may promote autoimmunity in several different disease models including ankylosing spondylitis, rheumatoid arthritis, and Sjögren syndrome.²⁵⁻²⁷ The link between ER stress and autoimmune responses has been proposed to be related to (a) misfolded proteins recognized as antigens by autoreactive immune cells, (b) dysregulation of immune-tolerance mechanisms in immune cells by ER stress, (c) ER stress-mediated death of cells releasing UPR-related autoantigens and/or neo-autoantigens, or (d) ER stress conferring a survival advantage to autoreactive cells.²⁷ Whether β -cell ER stress and a defective UPR can initiate autoimmunity or are induced as a consequence of chronic inflammation in T1D remains largely unknown.

ABERRANT UPR AND T1D

It has been suggested that disruption of ER homeostasis may contribute to β -cell dysfunction and diabetes. Consistent with this notion, misfolded insulin was shown to cause diabetes in both mouse models and humans.²⁸⁻³³ Moreover, in experimental animal models and in humans, mutations in genes critical for ER function result in β -cell failure and early onset, severe diabetes.³⁴⁻⁴² The UPR has been implicated in β -cell biology and pathogenesis of diabetes. A loss of function mutation of the *Perk* gene has been shown to cause permanent neonatal insulin-independent diabetes (Wolcott-Rallison syndrome) in humans and mice.^{34 36} Moreover, the IRE1 axis of the UPR has been shown to have a crucial function in insulin biosynthesis *in vitro* and *in vivo*.⁴⁰ X-box binding protein 1 deficiency in β cells causes modest hyperglycemia and glucose intolerance resulting from decreased insulin secretion from β cells.⁴² Finally, Wolfram syndrome, caused by an autosomal-recessive mutation in the *WSF1* gene, leads to

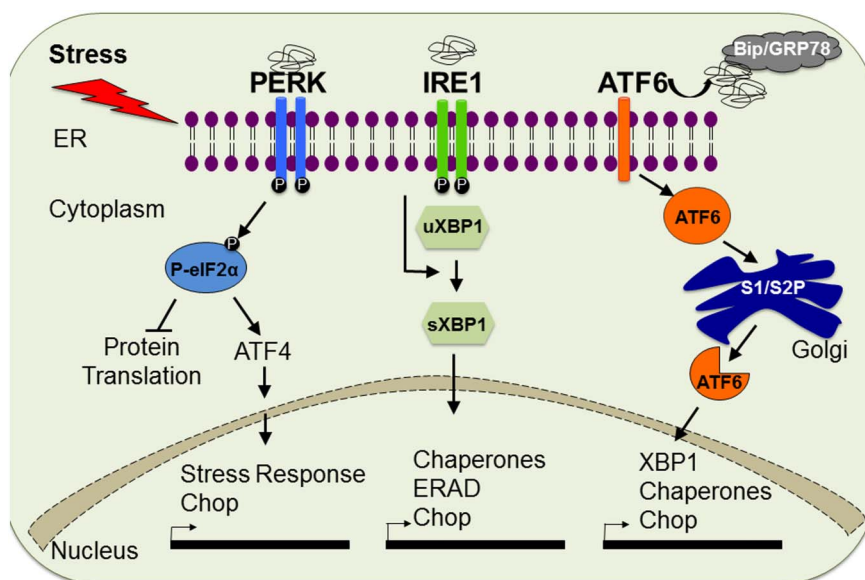


Figure 1 Unfolded protein response pathway. The UPR is triggered on ER stress and mediated by 3 sensors (PERK, IRE1/SXBP1, and ATF6) that are localized at the ER membrane.

nonautoimmune loss of β cells and insulindependent diabetes mellitus. In rodent and human cell lines, Wolfram syndrome 1 was shown to mitigate ER stress by negatively regulating ATF6.⁴³ The indication that the UPR may play a role in the pathogenesis of autoimmune diabetes came from recent reports that demonstrated the presence of some of the ER stress markers in inflamed islets of both diabetes-prone non-obese diabetic (NOD) mice⁴⁴ and patients with autoimmune diabetes.⁴⁵ In a recent study, we analyzed the protein levels of the 3 branches of the UPR in 2 different mouse models of T1D and in human patients with T1D in situ. This work has demonstrated that expression of adaptive UPR mediators sXBP1 and ATF6 were significantly diminished in β cells of NOD mice and human patients during the disease progression.⁴⁶ Interestingly, in a follow-up study, we showed that these markers were also downregulated in the β cells of both genetic and dietary mouse models of T2D, as well as in human T2D patients before the onset of hyperglycemia.⁴⁷ Thus, these data suggested that highly secretory β cells require an intact UPR and the loss of adaptive responses correlates with β -cell dysfunction and apoptosis.

Because, in experimental models of both T2D and atherosclerosis, chemical chaperones such as tauroursodeoxycholic acid (TUDCA) and phenyl butyric acid reduce ER stress and alleviate disease symptoms,^{48–50} we hypothesized that by increasing ER capacity, improving folding defects, or promoting the prosurvival and/or adaptive effects of the UPR, chemical chaperones might also protect pancreatic β cells in T1D. To test this hypothesis, we administered TUDCA to 2 different mouse models of T1D at the prediabetic stage and detected a striking reduction in diabetes with TUDCA treatment in these models. Histological analyses indicated a markedly decreased insulinitis and immune-mediated destruction in the islet area of TUDCA-treated mice. In line with reduced insulinitis, β -cell survival and islet architecture were preserved on TUDCA treatment. Immunophenotyping of the spleens, pancreata, and lymph nodes of TUDCA-treated animals revealed that systemic administration of TUDCA did not globally affect the relative representation of immune cell populations critically implicated in T1D while reducing the overall infiltration of islets. This could perhaps reflect decreased local antigen presentation or chemokine expression from islets,

migratory, and/or invasive defects of immune cells and/or potential alterations in T-cell subtypes. Better understanding of the mechanism(s) by which alleviation of ER stress leads to decreased immune cell infiltration remains to be elucidated.

To determine whether TUDCA's effects were altered in the genetic deficiency of the UPR, we induced diabetes in β -cell-specific ATF6 α -deficient mice and wild-type controls that were intercrossed with the RIP-LCMV-GP mice, a virally induced diabetes mouse model expressing the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) under control of the rat insulin promoter (RIP), in the presence or absence of TUDCA treatment. There was no difference in the diabetes incidence between the ATF6 α -deficient and control mice treated with vehicle. However, TUDCA's protective effects were completely lost in mice with β -cell-specific ATF6 α deficiency, demonstrating that the effect of TUDCA in T1D was dependent on the intact function of ATF6 in β cells. These data suggest that ATF6 acts as a prosurvival mediator of the UPR to maintain the function and survival of β cells in T1D and that TUDCA uses the ATF6 branch to induce the adaptive UPR and restore β -cell homeostasis (figure 2).

In addition to providing a direct link for the first time that the UPR plays an important role in β -cell survival, function, and T1D pathogenesis, this study provided proof of principle that the ER can be chemically targeted in an autoimmune disease to enhance its functional capacity. Although several interventions studies have been very successful in the NOD mouse model, these interventions failed to achieve similar effects in clinical studies. However, obtaining the same preventive effects in 2 different mouse models of T1D and demonstrating the similar UPR defects in human T1D patients, our study suggests that TUDCA, an agent that is already used safely in the clinic for liver diseases, might be useful as a preventative therapeutic agent for T1D because autoantibody screening allows detection of people at risk for T1D 2 to 3 years before the onset of the disease. Another interesting therapeutic possibility could involve using TUDCA as an adjuvant therapy. Although our studies indicate that administration of TUDCA after the onset of diabetes does not show any effect, whether it can be effective in combination with other immune modulating agents remains to be tested (figure 3).

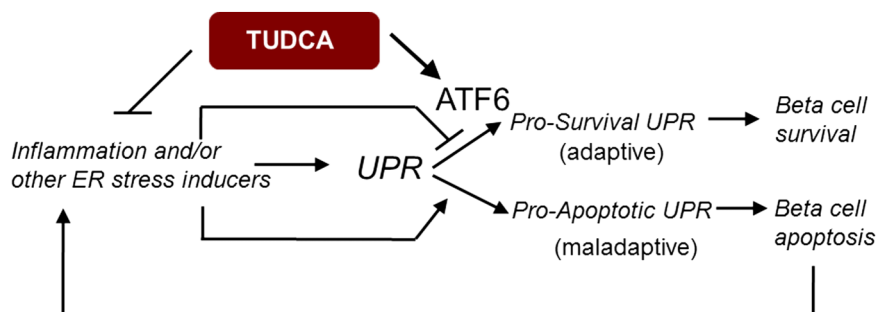
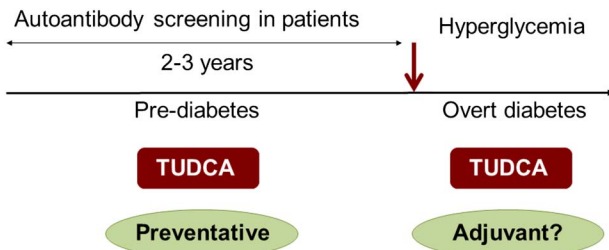


Figure 2 Tauroursodeoxycholic acid's effects in T1D. Inflammation or ER stress inducers can initiate the UPR. If ER stress is chronic and unresolvable, maladaptive UPR leads to β -cell apoptosis and death. This in turn can induce inflammation and ER stress. Tauroursodeoxycholic acid, by reducing inflammation and activating prosurvival UPR through ATF6 can give rise to β -cell survival and protection from diabetes.



- Used in clinic
- No safety issues
- Works in animal models

Figure 3 The translational possibilities of TUDCA. Tauroursodeoxycholic acid or other agents that could enhance ER function and restore the UPR might be used as preventative therapeutic agents for T1D. By autoantibody screening, people at high risk for T1D can be detected several years before the onset of the disease and this can be an ideal therapeutic window for the application of ER-modulating agents. In addition, TUDCA might be used as an adjuvant therapy and may provide better efficacy in combination with other immunomodulating agents.

FUTURE DIRECTIONS

The role of ER stress and the UPR in T1D pathogenesis is a newly emerging aspect of this field that is of great interest. Our recent work together with emerging data from other groups has established the importance of organellar stress and dysregulated adaptive UPR in the pathogenesis of autoimmune disease and raised the possibility that improving ER capacity and adaptive responses of the UPR in β cells might be a preventive strategy applicable to those who are at high risk for T1D. To date, several different strategies that focused mainly on immune modulation did not have a sustainable success in T1D clinical trials.⁵¹ Thus, enhancing β -cell ER function, and the adaptive UPR alone, or as a combination therapy might be a novel clinical approach. In addition to providing evidence that preserving in β cell's ER function can play a significant role in T1D pathogenesis, this study raised several interesting questions such as the following: (a) what are the functions of different branches of the UPR in T1D disease progression? (b) how do stressed β -cells crosstalk with immune cells? (c) are there biomarkers that can be indicative of β -cell ER stress or dysregulated UPR? and (d) do immune cells undergo ER stress and have altered function during T1D progression? We believe addressing these questions will greatly improve our current understanding of β -cell ER stress and the UPR and may be effectively incorporated into development of better therapeutic and preventive approaches for T1D.

Funding Supported in part by the National Center for Research Resources Grant R13 RR023236.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

- 1 Lehen A, Diana J, Zacccone P, et al. Immune cell crosstalk in type 1 diabetes. *Nat Rev Immunol* 2010;10:501–13.
- 2 Mathis D, Vence L, Benoist C. beta-Cell death during progression to diabetes. *Nature* 2001;414:792–8.
- 3 Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014;383:69–82.
- 4 van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 2011;91:79–118.

- 5 Donath MY, Storling J, Maedler K, et al. Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. *J Mol Med (Berl)* 2003;81:455–70.
- 6 Odegaard JI, Chawla A. Connecting type 1 and type 2 diabetes through innate immunity. *Cold Spring Harb Perspect Med* 2012;2:a007724.
- 7 Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nat Rev Drug Discov* 2014;13:465–76.
- 8 Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006;116:1793–801.
- 9 Brooks-Worrell B, Narla R, Palmer JP. Islet autoimmunity in phenotypic type 2 diabetes patients. *Diabetes Obes Metab* 2013;15(Suppl 3):137–40.
- 10 Reinehr T. Type 2 diabetes mellitus in children and adolescents. *World J Diabetes* 2013;4:270–81.
- 11 Itariu BK, Stulnig TM. Autoimmune aspects of type 2 diabetes mellitus - a mini-review. *Gerontology* 2014;60:189–96.
- 12 Minges KE, Whittemore R, Grey M. Overweight and obesity in youth with type 1 diabetes. *Annu Rev Nurs Res* 2013;31:47–69.
- 13 Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010;140:900–17.
- 14 Kars M, Yang L, Gregor MF, et al. Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. *Diabetes* 2010;59:1899–905.
- 15 Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004;306:457–61.
- 16 Bernales S, Papa FR, Walter P. Intracellular signaling by the unfolded protein response. *Annu Rev Cell Dev Biol* 2006;22:487–508.
- 17 Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011;334:1081–6.
- 18 Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007;8:519–29.
- 19 Størling J, Binzer J, Andersson AK, et al. Nitric oxide contributes to cytokine-induced apoptosis in pancreatic beta cells via potentiation of JNK activity and inhibition of Akt. *Diabetologia* 2005;48:2039–50.
- 20 Chambers KT, Unverferth JA, Weber SM, et al. The role of nitric oxide and the unfolded protein response in cytokine-induced beta-cell death. *Diabetes* 2008;57:124–32.
- 21 Oyadomari S, Takeda K, Takiguchi M, et al. Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. *Proc Natl Acad Sci USA* 2001;98:10845–50.
- 22 Osowski CM, Hara T, O'Sullivan-Murphy B, et al. Thioredoxin-interacting protein mediates ER stress-induced β cell death through initiation of the inflammasome. *Cell Metab* 2012;16:265–73.
- 23 Lu B, Nakamura T, Inouye K, et al. Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* 2012;488:670–4.
- 24 Lerner AG, Upton JP, Praveen PV, et al. IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab* 2012;16:250–64.
- 25 Panayi GS, Corrigan VM. BiP regulates autoimmune inflammation and tissue damage. *Autoimmun Rev* 2006;5:140–2.
- 26 Bläss S, Union A, Raymackers J, et al. The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. *Arthritis Rheum* 2001;44:761–71.
- 27 Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 2008;8:663–74.
- 28 Støy J, Edghill EL, Flanagan SE, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci U S A* 2007;104:15040–4.
- 29 Wang J, Takeuchi T, Tanaka S, et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. *J Clin Invest* 1999;103:27–37.
- 30 Izumi T, Yokota-Hashimoto H, Zhao S, et al. Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. *Diabetes* 2003;52:409–16.
- 31 Colombo C, Porzio O, Liu M, et al. Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. *J Clin Invest* 2008;118:2148–56.
- 32 Molven A, Ringdal M, Nordbø AM, et al. Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. *Diabetes* 2008;57:1131–5.
- 33 Polak M, Dechaume A, Cavé H, et al. Heterozygous missense mutations in the insulin gene are linked to permanent diabetes appearing in the neonatal period or in early infancy: a report from the French ND (Neonatal Diabetes) Study Group. *Diabetes* 2008;57:1115–9.

- 34 Zhang P, McGrath B, Li S, *et al.* The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. *Mol Cell Biol* 2002;22:3864–74.
- 35 Harding HP, Zhang Y, Bertolotti A, *et al.* Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* 2000;5:897–904.
- 36 Delépine M, Nicolino M, Barrett T, *et al.* EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. *Nat Genet* 2000;25:406–9.
- 37 Oyadomari S, Yun C, Fisher EA, *et al.* Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. *Cell* 2006;126:727–39.
- 38 Scheuner D, Song B, McEwen E, *et al.* Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell* 2001;7:1165–76.
- 39 Ladiges WC, Knoblaugh SE, Morton JF, *et al.* Pancreatic beta-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. *Diabetes* 2005;54:1074–81.
- 40 Lipson KL, Fonseca SG, Ishigaki S, *et al.* Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab* 2006;4:245–54.
- 41 Song B, Scheuner D, Ron D, *et al.* Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. *J Clin Invest* 2008;118:3378–89.
- 42 Lee AH, Heidtman K, Hotamisligil GS, *et al.* Dual and opposing roles of the unfolded protein response regulated by IRE1alpha and XBP1 in proinsulin processing and insulin secretion. *Proc Natl Acad Sci USA* 2011;108:8885–90.
- 43 Fonseca SG, Ishigaki S, Osowski CM, *et al.* Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. *J Clin Invest* 2010;120:744–55.
- 44 Tersey SA, Nishiki Y, Templin AT, *et al.* Islet β -cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. *Diabetes* 2012;61:818–27.
- 45 Marhfour I, Lopez XM, Lefkaditis D, *et al.* Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. *Diabetologia* 2012;55:2417–20.
- 46 Engin F, Yermalovich A, Nguyen T, *et al.* Restoration of the unfolded protein response in pancreatic beta cells protects mice against type 1 diabetes. *Sci Transl Med* 2013;5:211ra156.
- 47 Engin F, Nguyen T, Yermalovich A, *et al.* Aberrant islet unfolded protein response in type 2 diabetes. *Sci Rep* 2014;4:4054.
- 48 Erbay E, Babaev VR, Mayers JR, *et al.* Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nat Med* 2009;15:1383–91.
- 49 Ozcan U, Yilmaz E, Ozcan L, *et al.* Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 2006;313:1137–40.
- 50 Engin F, Hotamisligil GS. Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases. *Diabetes Obes Metab* 2010;12:108–15.
- 51 Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 2010;464:1293–300.