Endoplasmic reticulum (ER) stress & diabetes

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The endoplasmic reticulum (ER) is a central organelle entrusted with lipid synthesis, protein folding and protein maturation. It is endowed with a quality control system that facilitates the recognition and targeting of aberrant proteins for degradation. When the capacity of this quality control system is exceeded, a stress response (ER stress) is switched on. Prolonged stress leads to apoptosis and may thus be an important factor in the pathogenesis of many diseases. A complex homeostatic signaling pathway, known as the unfolded protein response (UPR), has evolved to maintain a balance between the load of newly synthesized proteins and the capacity of the ER to aid in their maturation. Dysfunction of the UPR plays an important role in certain diseases, especially those involving tissues dedicated to extracellular protein synthesis. Diabetes is an example of such a disease, since pancreatic β-cells depend on efficient UPR signaling to meet the demands for constantly varying levels of insulin synthesis. Recent studies have indicated that the importance of the UPR in diabetes is not restricted to the β-cell but also to tissues of peripheral insulin resistance such as liver and adipose tissue. Better understanding of the basic mechanisms of ER stress and development of insulin resistance/type 2 diabetes is pivotal for the identification of newer molecular targets for therapeutic interventions.

Key words Diabetes - ER stress - insulin resistance - UPR

Diabetes mellitus (DM) is a metabolic disorder characterized by varying or persistent hyperglycaemia due to reduced insulin action. The underlying defect may be decreased secretion of insulin, its impaired signaling or both. Type 1 diabetes is known to result from an excessive loss of pancreatic β-cells while type 2 diabetes is a consequence of β-cell dysfunction[1]. Diabetes is a multifactorial disorder and several different mechanisms have been implicated in the development of the disease. But the precise molecular events underlying this phenotype still remain obscure. Among other important causes, autoimmune and inflammatory processes have been reported to selectively disrupt β-cells and cause insulin deficiency and hyperglycaemia and

subsequent type1 diabetes. Insulin resistance, often associated with obesity and physical inactivity, is a major factor in the progression of type2 diabetes. Accumulating evidence suggests that apoptosis may be the main mode of β-cell death in both types of diabetes[2,3]. Recent studies point to the role of the endoplasmic reticulum (ER) in the sensing and transduction of apoptotic signals. This review focuses on ER stress and its involvement in the development of diabetes mellitus.

Endoplasmic reticulum - A specialized protein folding compartment

ER is the first compartment of the secretory pathway in eukaryotic cells. The lumen of the ER provides a specialized environment for the post-translational modification and folding of secreted, transmembrane and resident proteins of various compartments. The ER is a membrane enclosed compartment with a luminal space that is topologically equivalent to the extracellular space. Proteins destined for the ER bear a predominantly hydrophobic signal sequence and guided by it, traverse the ER membrane either co- or post-translationally through the Sec61p complex[4,5]. This sets into motion a complex folding pathway which finally helps the protein fold into its native conformation. While a number of post-translational modifications including lipidation, hydroxylation, oligomerization, *etc*., occur in the ER, disulphide oxidation and N-linked glycosylation are among the general modifications that are common to the majority of secreted proteins. The ER lumen has a highly oxidizing environment and facilitates disulphide bond formation. Disulphide bonds are important for the stability and function of a large number of proteins. Disulphide oxidation in the ER is catalyzed by protein disulphide isomerases (PDI). Similarly, glycosylation is a vital part of protein folding and serves several important purposes; the hydrophilic nature of carbohydrates facilitates greater solubility of glycoproteins and defines the attachment area for the surface of the protein. The large hydrated volume of the oligosaccharides helps shield the attachment area from surrounding proteins and more importantly, the oligosaccharides interact with the peptide backbone and stabilize its conformation[6]. The most important role for glycosylation seems to be in its ability to aid the 'Quality Control' apparatus of the ER. While properly folded and assembled proteins are cleared for exit from the ER and progress down the secretory pathway, incompletely folded proteins are retained to complete the folding process or to be targeted for degradation in a process termed quality control[7,8]. The glycosylation status is monitored by a lectin machinery, the calnexin-calreticulin cycle, an arm of the quality control apparatus of the ER[9]. This determines whether the protein is exported to the Golgi or targeted for degradation. In addition to these lectins, ER protein folding machinery consists of two other classes of proteins, foldases, (enzymes catalyzing protein folding) and molecular chaperones (proteins that facilitate protein folding by preventing aggregation). BiP/GRP78, a molecular chaperone that belongs to the HSP70 class of chaperones, plays a vital role in the recognition of unfolded proteins and therefore, is a key player in ER stress[7,8].

In addition to its role in protein folding, ER is also the site of sterol and lipid synthesis[10] and also serves as a cellular Ca^{2+} store[11]. In principle, disruption of any of these processes could lead to ER stress but currently there is very little known in this regard. Traditionally though, the major focus has been on ER stress caused by disruption of protein folding.

Unfolded protein response - A stress buster for cells

Cells depending on their type and physiological state would be required to process varying loads of ER client proteins and they adapt to this variation by modulating both the capacity of their ER and the synthesis of client proteins. Disequilibrium between this ER load and folding capacity is referred to as ER stress. The ability to adapt to physiological levels of ER stress is important to cells, especially to

professional secretory cells like the insulin producing β-cells. An increase in the synthesis of client proteins triggers ER stress as do several pathophysiological states like hypoxia, exposure to natural and experimental toxins and a variety of mutations that hinder proper folding of client proteins[12,13]. Studies have established that expression of mutant, folding-incompetent proteins causes ER stress and elicits an ER stress response, called the unfolded protein response (UPR)[14-17]. The initial intent of the UPR is adaptation and restoration of the normal ER function. The failure of such adaptative mechanisms leads to alarm signaling and finally to cell suicide, usually in the form of apoptosis, as a last resort to do away with dysfunctional cells. This is the biochemical basis for many ER storage diseases[18,19]. Activation of UPR can be brought about by the exhaustion of the capacity of the complex ER resident protein folding machinery, *e.g.*, overexpression of factor VIII[20], antithrombin III[21]. Even in some physiological conditions, the demand on the ER resident protein folding machinery exceeds its capacity, *e.g.*, differentiation of B-cells into plasma cells[22]. Some of the other conditions known to trigger ER stress and activate UPR are glucose deprivation, aberrant Ca^{2+} regulation, viral infections, *etc*[23].

The adaptive mechanisms employed to combat ER stress and restore normal function include upregulation of the folding capacity of the ER and downregulation of its biosynthetic capacity[24]. At least four distinct responses that constitute the UPR have been identified so far[25] (Fig. 1). The first response involves upregulation of the genes encoding ER chaperone proteins including BiP/GRP78 and GRP94. This helps increase protein
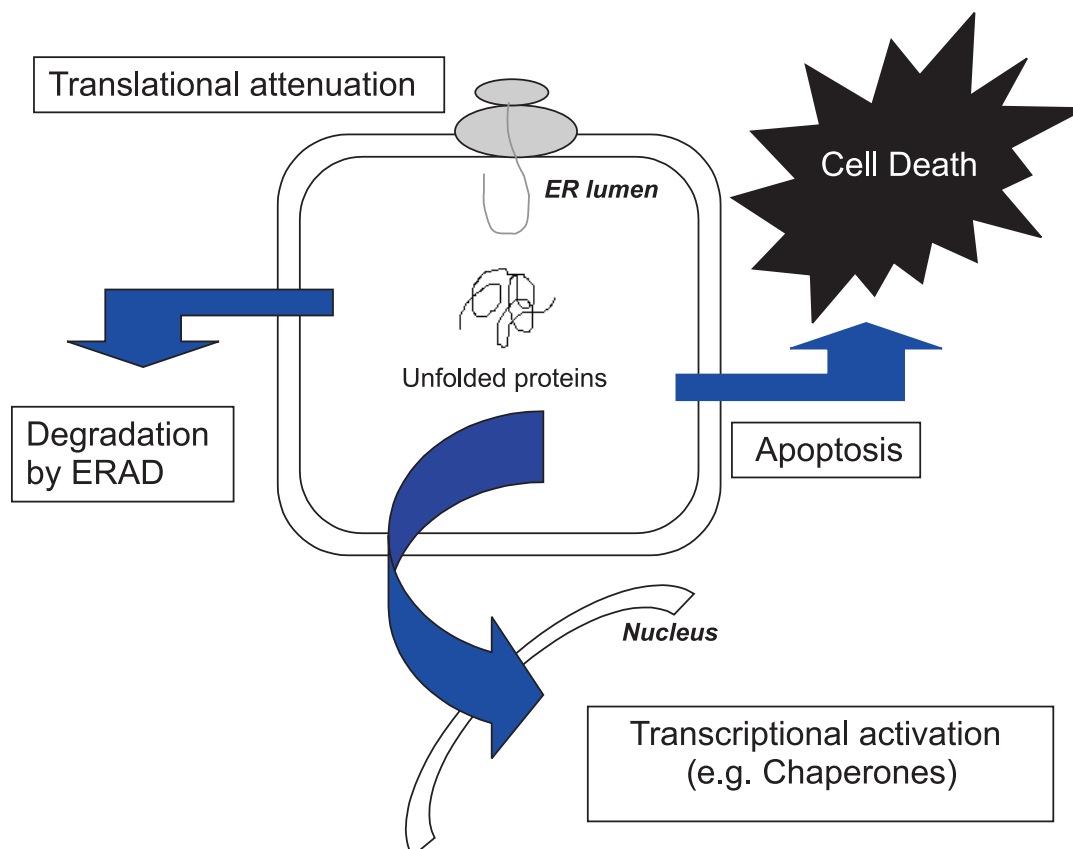

Fig. 1. The ER stress response. The unfolded protein response involves four distinct processes: (*i*) increase in the protein folding capacity through transcriptional induction of ER chaperones; (*ii*) reduction of the biosynthetic load by translational attenuation; (*iii*) degradation of the misfolded proteins by ERAD; and (*iv*) apoptosis, as a last resort, to eliminate infected cells and maintain homeostasis. ERAD, endoplasmic reticulum-associated degradation.

folding activity and prevents protein aggregation. The second response consists of translational attenuation to reduce the biosynthetic load and thus prevents further accumulation of unfolded proteins. The third response is the degradation of misfolded proteins by ER-associated degradation (ERAD). Terminally misfolded proteins which cannot be refolded in the ER are retro-translocated into the cytosol and degraded by the proteasomes. When these responses fail to remedy the ER stress, apoptosis, the fourth response is resorted to, in a bid to eliminate unhealthy or infected cells and maintain proper development and differentiation.

Transduction of the UPR signal

Transduction of the UPR signal across the ER membrane is carried out by three transmembrane proteins (Fig. 2) (*i*) IRE1 (inositol requiring 1)[26], a type I transmembrane protein, (*ii*) PERK [double-stranded RNA-activated protein kinase (PKR) - like endoplasmic reticulum kinase][27]; and (*iii*) ATF6 (activating transcription factor 6)[28], a type II transmembrane protein.

The ER luminal domains of IRE1 and PERK share a small degree of homology conserved
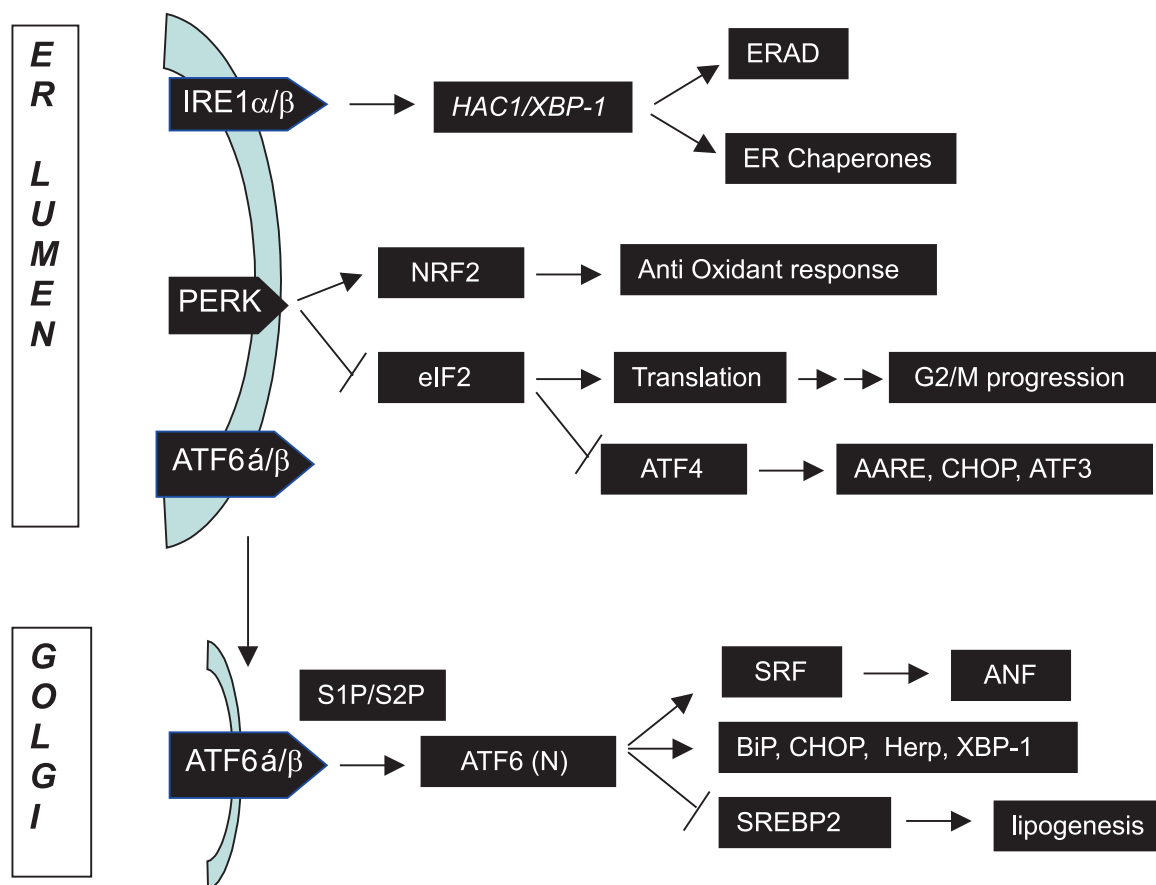

Fig. 2. Overview of the ER stress signaling pathways. Activation of protective responses by UPR involves signal transduction through the IRE1, PERK and ATF pathways. The IRE1 pathway regulates chaperone induction and ERAD in response to ER stress. The PERK pathway through its actions on eIF2 and NRF2 influences general translation and contributes to cell survival during ER stress. ATF6 acts as a transcription factor and regulates important targets such as *BiP*, *XBP-1* and *CHOP*. AARE, amino acid response element; ANF, atrial natriuretic factor; ATF, activating transcription factor; CHOP, C/EBP homologous protein; HAC1, homologous to ATF/CREB1; NRF2, nuclear factor erythroid 2-related factor 2; SRF, serum response factor.

throughout all eukaryotes and act as ER stress regulated homodimerization domains[29]. BiP remains associated with the luminal domains of IRE1 and PERK in their inactive state[30]. Upon ER stress, the large excess of unfolded proteins in the ER lumen necessitates BiP dissociation and the resultant oligomerization of IRE1 and PERK leads to activation of the proximal signal transducers. ATF6, identified by Mori and colleagues[28] is also regulated by BiP, albeit in a slightly different way. BiP binds to the Golgi localization signals (GLS) on ATF6 and thus retains it in the ER. During ER stress, BiP dissociates, causing ATF6 to transport to the Golgi and get activated[31].

IRE1: Chaperone induction and ERAD, in response to ER stress, are regulated by the IRE1 pathway. IRE1, in addition to its ER luminal dimerization domain, consists of cytosolic kinase and endoribonuclease domains. After dissociation from BiP, IRE1 oligomerizes and activates its RNase domain by autophosphorylation[32,33]. In yeast, this eventually leads to splicing of its substrate, *HAC1* mRNA[34]. Spliced Hac1p binds to the unfolded protein response element (UPRE) and upregulates ER chaperone genes[35]. There are two mammalian IRE1 proteins and both participate in ER stress signaling. IRE1α is broadly expressed while IRE 1β is selectively expressed in foregut-derived epithelium. It turns out that ER stress mediated activation of mammalian IRE1 results in the splicing of the X-box binding protein 1(XBP-1) mRNA[36]. XBP-1 is a transcription factor expressed at high levels in cells actively engaged in protein secretion. It controls genes containing a CRE (cAMP response element) - like element[37]. Studies in yeast and *Arabidopsis thaliana* have revealed that the IRE1 pathway co-ordinates multiple aspects of the secretory pathway such as chaperone induction, upregulation of ERAD genes, membrane biogenesis and ER-quality control[38,39]. In mammalian cells, XBP-1 regulates a subset of ER-resident molecular chaperones[40]. These findings suggest that ER stress, acting through the IRE1 and

XBP-1 dependent signaling pathway, upregulates the secretory apparatus in cells and defective signaling in this pathway would affect professional secretory cells such as islet β cells.

PERK: PERK pathway, on the other hand, plays a major role in the translational attenuation and subsequently the regulation of protein synthesis in response to ER stress[41]. This is brought about by a decreased activity of the eukaryotic initiation factor 2 (eIF2) complex, which normally recruits charged initiator methionyl tRNA to the 40S ribosomal unit. Phosphorylation of eIF2 complex α subunit on serine 51 has evolved as a major mechanism for reducing translation initiation and protein synthesis in eukaryotes. ER stress mediated phosphorylation of eIF2α is carried out by PERK. As IRE1, *PERK* on dissociation from BiP, oligomerizes and phosphorylates its substrate proteins, eIF2α[41] and the transcription factor, Nrf2[42]. Phosphorylation of eIF2α shuts off general translation while that of Nrf2 contributes to survival of cells during ER stress. While phosphorylation of eIF2α shuts off majority of the protein synthesis, it also seeks to de-repress, under stress conditions, some mRNAs that are otherwise basally repressed. ATF4 mRNA, for example, is activated during ER stress and consequently, target genes including that of BiP, XBP-1 and CHOP are activated. Thus PERK plays a major role not only in the translational attenuation during ER stress but also in selective stress-induced gene expression[43].

ATF6: Two similar transcription factors, ATF6α and ATF6β, exist in mammals. These are activated by regulated intramembrane proteolysis in ER stressed cells. Normally ATF6 is retained in an inactive form by association with ER membranes. In this case, BiP regulates the activity of two independent and redundant Golgi localization sequences, GLS1 and GLS2. BiP binds to GLS1 but not to GLS2 resulting in a constitutive translocation of ATF6 to the Golgi and its activation[31,44]. Additionally, ATF6 is retained in the ER by its interaction with calreticulin. ER

stress results in underglycosylation of ATF6, abrogates this interaction and hastens its translocation to the Golgi[45]. Thus, both quality control mechanisms of the ER, namely recognition of unfolded proteins by BiP and the calnexin/calreticulin cycle regulate the activity of ATF6. On dissociation from BiP, ATF6 translocates to the Golgi complex where it is cleaved by Site-1 and Site-2 proteases (S1P and S2P)[46] to release the cytosolic N-terminal portion, a basic leucine zipper (bZIP) transcription factor. ATF6 binds to the ATF/CRE element and to the ER stress response elements I and II and regulates important targets such as *BiP*, *XBP-1*, and *CHOP*[47]. Also, ATF6 forms a complex with the transcription factor sterol response element binding protein 2 (SREBP2) to repress transcription and thus counters the lipogenic effects of SREBP2[48].

ER stress mediated apoptosis

Apoptosis in response to ER stress is a response specific for metazoans. When severe and prolonged ER stress extensively impairs the ER functions, apoptosis is necessary not only for removing the cells that threaten the integrity of the organism but also for proper development and differentiation. Apoptosis is also the least well understood ER stress response pathway with so many mechanisms and so little clarity. Broadly, the apoptotic pathways triggered by ER stress fall into three categories. The first is the transcriptional induction of the gene for CHOP/GADD153, a member of the C/EBP family of transcription factors[49]. While CHOP is barely detected under physiological conditions, it is strongly induced in response to ER stress[50]. Studies utilizing strategies of overexpression and targeted disruption of CHOP gene have demonstrated that CHOP promotes apoptosis in response to ER stress[51]. Transcriptional activation of the CHOP gene is mediated by all the three ER stress transducers, namely PERK, IRE-1 and ATF6. The downstream targets of CHOP leading to apoptosis are still unclear. The second apoptotic pathway involves activation of the c-Jun N-terminal kinase (JNK) pathway. In response to ER stress, activated IRE-1 recruits

tumour necrosis factor receptor-associated factor 2 (TRAF-2) and apoptosis signal-regulating kinase 1 (ASK-1) to form Ire-1-TRAF2-ASK1 complex which then activates JNK and triggers cell death[52]. Also, c-Jun N-terminal inhibitory kinase (JIK) associates with IRE1 and promotes phosphorylation and association of TRAF2 with IRE1. The third apoptotic pathway in response to ER stress depends on the activation of ER-localized cysteine protease, caspase -12[53]. Perturbation of ER Ca^{2+} pools in response to ER stress activates calpain in the cytosol which in turn converts procaspase-12 to its active form[54]. Caspase-12 then initiates a caspase cascade culminating in apoptosis and cell death. Surprisingly, this pathway seems to be independent of Apaf-1 and mitochondrial cytochrome-c release.

ER stress & diabetes

Akita mouse - Animal model of ER stress mediated diabetes: The earliest clue to the involvement of ER stress in diabetes came from studies on Akita mouse. The Akita mouse is a spontaneously diabetic model, characterized by progressive hyperglycaemia with reduced β-cell mass without insulitis or obesity[55]. Genetic analyses revealed that a mutation in the insulin 2 gene (*Ins2*) (Cys96Tyr) is responsible for the diabetic phenotype in this mouse[56]. This mutation disrupts a disulphide bond formation between the A chain (A7) and the B chain (B7) of proinsulin, thereby inducing a drastic conformational change. The mutant proinsulin is retained in the ER and according to a recent study[57] gets degraded by ERAD on high ER stress. HRD1 (for HMG-CoA reductase degradation), a component of the ERAD system has been shown to be upregulated in the pancreatic islets of Akita mice which causes enhanced degradation of the misfolded insulin. However loss of insulin production by the mutant allele alone is unlikely to have a major impact since rodents have two insulin genes (*Ins1* and *Ins2*) and the loss of both copies of *Ins2* has no metabolic consequences[58]. Further investigations have revealed that progressive hyperglycaemia in the mouse was accompanied by elevated levels of ER stress markers such as BiP and

CHOP. Since proinsulin is a major ER client protein in the pancreatic β-cells, it is likely that its malfolding is causing ER stress. Oyadamari *et al*[59] have shown that when the Akita mutation was introduced into a *CHOP*^{-/-} background, islet cell destruction and hyperglycaemia were delayed on onset. The *CHOP* knockout reduced cell death by ER stress of any cause, suggesting that ER stress plays an important role in the pathogenesis of islet cell dysfunction in Akita mice. It is also clear from these studies that CHOP activation is not the only mechanism since *CHOP* knockout simply delayed the onset and did not prevent the disease altogether.

Mutations in PERK gene: Clues to involvement of ER stress in diabetes: Another significant piece of evidence implicating ER stress in the pathology of diabetes was discovered by Delephine and colleagues in their study of Wolcott-Rallison syndrome (WRS)[60]. WRS is a rare, autosomal recessive disorder characterized by early infancy onset diabetes mellitus. Mutations in the *EIF2AK3* gene were found to be the underlying cause of this disorder. *EIF2AK3* codes for the pancreatic ER kinase (PERK), one of the major ER stress transducers. This study however, did not address the pathophysiological basis for the phenotype. More insights into this were provided by Harding *et al*[61] through their studies on *Perk*^{-/-} mice. The same authors had shown earlier that *Perk*^{-/-} cells were unable to phosphorylate eIF2α and attenuate translation in response to ER stress[62]. Also, the absence of PERK rendered these cultured cells hypersensitive to toxins that interfered with normal protein folding in the ER. *Perk*^{-/-} mice developed a clinical syndrome similar to that seen in WRS patients. Though born with nearly normal islets of Langerhans, these mice showed progressive destruction of B-cells in the first few weeks of life. When islets from pre-diabetic *Perk*^{-/-} mice were explanted and placed in culture, they synthesized, processed and secreted the mature insulin in a normal manner. When the cultures were switched to high glucose, the mutant islets increased insulin production more vigorously than islets isolated from wild type mice. Glucose, since it stimulates insulin production, promotes some ER stress by imposing a load on the folding and protein processing machinery of the ER. In the wild type mouse, this induces a rectifying pathway consisting of PERK activation and subsequent reduction of protein synthesis. Since this important mechanism is lost in *Perk*^{-/-} mice, protein synthesis becomes unresponsive to this stress and there is accumulation of unfolded client proteins (*e.g.*, proinsulin) in excess of the chaperone reserve. The authors speculate that this might lead to production of novel toxic configurations of proteins that may damage the islets. True to this speculation, electron microscopy has revealed an accumulation of electron dense material in the ER and distorted organelle morphology in the *Perk*^{-/-} islets[61]. These findings emphasize the role of loss of translational control in the pathophysiology of *PERK* mutant islet cells. As discussed earlier, *PERK* through its action on eIF2α, controls not only translation but also activates certain stress induced genes. So it is possible that reduced activity of these survival genes may also contribute to the death of β cells both in *Perk*^{-/-} mice and in the WRS patients. There have been several other reports in recent times that have supported the importance of translational control in preserving ER function in β-cells and maintaining glucose homeostasis[63-65]. Scheuner *et al*[64] have shown that eIF2α phosphorylation is an important mechanism for compensation and prevention of diet-induced diabetes. This becomes necessary to prevent ER dysfunction and impaired insulin secretion in conditions of insulin demand. These authors have studied glucose homeostasis in *Eif2s1*[tm1Rjk] mutant mice, which have an alanine substitution at Ser 51 of eIF2α and reported that profound glucose intolerance results from reduced insulin secretion. This is also accompanied by abnormal distension of the ER lumen, defective trafficking of proinsulin and a reduced number of insulin granules in β cells.

Obesity, ER stress and diabetes: ER also plays a crucial role in the regulation of cellular responses of

insulin. A recent report by Ozcan *et al*[66] throws more light on the link between obesity, ER stress, insulin action and type 2 diabetes. Using cell culture and mouse models, the authors have shown that obesity causes ER stress. They observed an elevation of several biochemical indicators of ER stress, namely PERK and eIF2α phosphorylation, c-Jun N-terminal kinase (JNK) activity and BiP expression in liver and adipose tissues of obese animals compared to their lean counterparts. ER stress led to a significant increase in JNK-mediated serine phosphorylation of insulin receptor-substrate 1(IRS-1) and thereby inhibited insulin action. IRS-1 is a substrate for insulin receptor tyrosine kinase, and serine phosphorylation of IRS-1, particularly mediated by JNK, reduces insulin receptor signaling[67]. The work by Ozcan *et al*[66] also provided evidence for the involvement of an IRE-1α and JNK-dependent protein kinase cascade in the inhibition of insulin action induced by ER stress. Further, employing XBP-1 gain- and loss-of-function cellular models in parallel with *XBP-1*^{+/-} mice, they have proved that loss of XBP-1 predisposes to diet-induced peripheral

insulin resistance and type 2 diabetes. Based on all these observations, the authors postulated that ER stress underlies the emergence of the stress in obesity and the integrated deterioration of systemic glucose homeostasis resulting in type 2 diabetes (Fig. 3).

Involvement of ER stress in diabetes: Other significant observations

ER stress and Wolfram syndrome: Studies on Wolfram syndrome, a rare autosomal recessive disorder, have lent support to the role of ER stress in diabetes. Wolfram syndrome is also known as DIDMOAD, the acronym for diabetes insipidus, diabetes mellitus, optic atrophy and deafness that summarizes the main clinical features of this disorder. Loss-of-function mutations in the *WFS1* gene, which codes for an ER transmembrane protein Wolframin, have been linked to this disorder[68]. Recent studies have identified WFS-1 as a novel component of the UPR[69] and that WFS-1 deficiency leads to apoptosis specifically in pancreatic β cells[70].
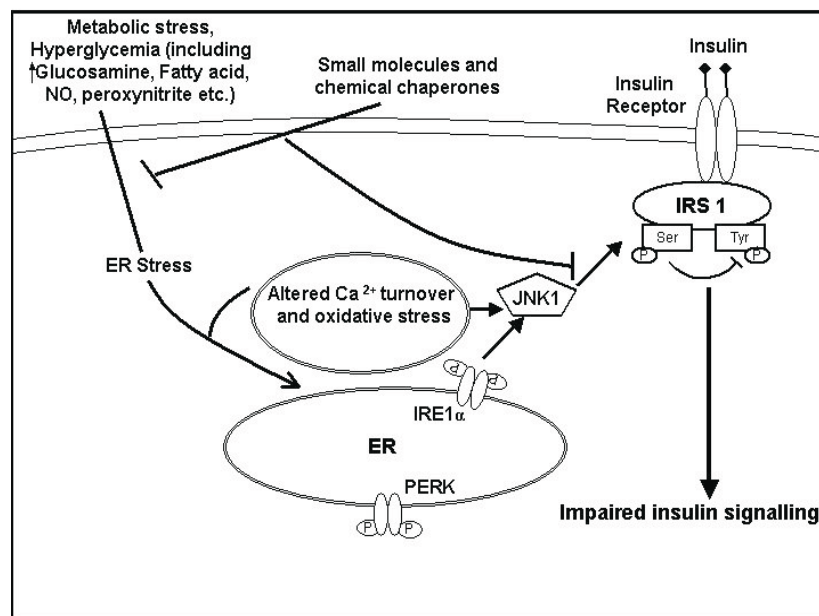

Fig. 3. ER stress and impaired insulin action. Metabolic stress and/or hyperglycaemia are associated with triggering of ER stress and UPR activation (enhanced phosphorylation of PERK, IRE 1 and activation of JNK1). Very often, disturbed Ca^{2+} homeostasis and altered redox signaling appear to be the common denominators of augmentation of JNK1. Insulin signaling is attenuated in part by the serine phosphorylation by JNK1 which prevents the physiological tyrosine phosphorylation of IRS-1. Small molecule drugs and chemical chaperones appear to attenuate ER stress, reduce JNK1 activity and thereby improve insulin action.

Increased hexosamines and ER stress: There is accumulating evidence to suggest that high glucose levels in type 2 diabetes lead to increased production of intracellular glucosamine via the hexosamine pathway[71]. Studies have indicated that glucosamine might induce ER stress that results in cholesterol accumulation in aortic smooth muscle cells, monocytes, and hepatocytes and thus could contribute to the development of atherosclerosis[72]. In a recent study, the possibility of convergence of ER stress and hexosamine pathways in the pathogenesis of insulin resistance in L6 skeletal muscle cells[73] was suggested. It was indicated that ER stress induced by glucosamine in early hours, could have an adaptive or rescue component during which time there is upregulation of ER chaperones. However, sustained glucosamine-induced ER stress appears to induce a lethal effect by way of turning on apoptotic events and negative regulation of insulin signaling as revealed by inhibition of insulin-stimulated glucose transport in L6 cells[73].

Nitric oxide and ER stress: Nitric oxide (NO), one of the important effectors of β-cell death in type 1 diabetes and vascular complications in type 2 diabetes, has been shown to exert its effects by triggering ER stress[74]. Oyadomari *et al*[75] have shown that NO depletes ER Ca^{2+}, causes ER stress and leads to apoptosis. NO-depletion of ER Ca^{2+} has been claimed to occur either by inhibition of Ca^{2+} uptake from cytosol through SERCA (tyrosine nitration) or by activation of Ca^{2+} release to cytosol through RyR (S-nitrosylation). Relatively low concentrations of NO induce apoptosis, at least in some types of cells including pancreatic cells[75] and macrophages[76], even though severe DNA damage did not occur in these models. Depletion of ER Ca^{2+} and activation of ER stress pathway including ATF6 activation and CHOP/GADD153 induction was also detected in those cells treated with NO. In addition, pancreatic islets and peritoneal macrophages from CHOP knockout mice showed resistance to NO-induced apoptosis. Peroxynitrite, a potent oxidant generated by the reaction of NO with superoxide, has also been shown to induce its proatherogenic effects through induction of ER stress[77].These studies attest to the involvement of ER stress pathway in NO-induced apoptosis.

ER stress induction by fatty acids: Saturated fatty acid (FA) overload in non-adipose tissues under certain conditions compromises the cellular capacity to store these FAs as triglycerides or to oxidize them for energy. This FA overload can lead to the production of reactive oxygen species (ROS) which can in turn induce ER stress. It has been shown that both free fatty acids and cytokines induce pancreatic β-cell apoptosis through ER stress[78,79]. Borradaile *et al*[80] have suggested that palmitate can be rapidly incorporated into complex lipids in the ER membrane and this increased saturation of ER membrane lipids could lead to dramatic impairment of the structure and integrity of the organelle. Both oxidative stress and altered ER composition and integrity could result in the release of ER calcium (Ca^{2+}) stores, triggering apoptotic cell death via the mitochondria. It has also been shown recently that ER stress is central to cholesterol-induced apoptosis in macrophages[81]. Taken together, these results seem consistent with a more general paradigm in which perturbations of cellular lipid metabolism can result in a death response initiated by events occurring at the ER. Thus, the findings from these studies suggest that in complications arising out of lipid metabolic disorders, ER could well be a proximal target for therapies aimed at improving cellular function.

ER stress in diabetes: Roles of other resident chaperones

Some recent studies have pointed to the role of certain ER resident proteins and chaperones in ER stress mediated diabetes. Ozawa *et al*[82] have shown that oxygen regulated protein (ORP150), a molecular chaperone located in the ER, plays an important role in insulin sensitivity and could be a potential target for the treatment of diabetes. They have observed that systemic expression of ORP150 in Akita mice improved insulin intolerance and enhanced glucose uptake, accompanied by suppression of oxidized protein. Another report has indicated that P58 (IPK),

an ER molecular chaperone might function as a signal for the downregulation of ER-associated proteins involved in the initial ER stress response, thus preventing excessive cell loss by degradation pathways[83,84]. P58 (IPK) is known to be induced during ER stress and functions as a negative feedback component to inhibit eIF-2α signaling and attenuate the later phases of the ER stress response. It has been shown that insulin deficiency observed in the absence of P58 (IPK), mimicked β-cell failure that is commonly seen in type1 and late stage type 2 diabetes patients.

Chemical chaperones and hope for newer therapies

In the past, low molecular weight compounds have been used to increase thermal stability and to reverse the improper localization and aggregation of proteins associated with human disease. These molecules, originally known as osmolites, have now been redefined as 'chemical chaperones'. Chemical chaperones come in two flavours, specific and nonspecific. Specific chaperones (V2 receptor antagonists) function as competitive inhibitors or substrate analogs of specific enzyme and proteins. Nonspecific chemical chaperones [such as phenyl butyric acid (PBA), and taurine-conjugated ursodeoxycholic acid (TUDCA)] have the general property of improving folding and trafficking without targeting a specific mutant protein.

Recently, Ozcan et al[85] showed that PBA and TUDCA attenuate the induction of ER stress by tunicamycin (a glycosylation inhibitor and UPR activator) in FaO rat hepatoma cells and mouse embryonic fibroblasts. When they extended their studies to the leptin-deficient ob/ob mouse model of obesity and diabetes, administration of PBA and TUDCA to diabetic ob/ob mice resulted in a rapid normalization of fasting blood glucose levels, improved glucose tolerance and reduced hyperinsulinaemia, consistent with improved insulin action. In addition, PBA and TUDCA attenuated ER stress activation (reduced PERK and IRE1 phosphorylation) and JNK activity (as indicated in Fig. 3), and improved insulin signaling in liver and adipose tissue of the diabetic ob/ob mice. Hyperinsulinaemic-euglycaemic clamp studies support both improvements in hepatic glucose production and glucose disposal in muscle and adipose tissue following chemical chaperone application. The findings of Ozcan et al[85] have created new hopes in that chemical chaperones might have therapeutic potential for the treatment of insulin resistance and type 2 diabetes.

Conclusion

Concrete evidence now exists to implicate ER stress in the development of diabetes. Several reports seem to suggest that the insulin secreting β-cell may be especially sensitive to the adverse effects of perturbed ER function. It is possible that prolonged ER stress might contribute to the degradation of β-cell function that precedes metabolic decompensation in the insulin-resistant subject. While ER stress in adipose tissue and liver, the peripheral tissues of insulin resistance, has been widely studied, it is still important to understand the events that occur in skeletal muscle, the major site of glucose disposal. The signs of ER stress found in liver and adipose tissue of obese and high-fat-diet fed mice indicate that the metabolic abnormalities associated with obesity and unhealthy diet may also cause ER stress *in vivo* in humans. Moreover, signaling events in the death pathway downstream of ER stress, though attractive as key therapeutic targets, still remain poorly understood and warrant in-depth analyses. Given the perceived importance of ER damage-induced cell death in diabetes and associated disorders, there is much hope in the development of small molecules for therapeutic usage targeting upstream (ER chaperones) and downstream events (pro- and anti-apoptotic signaling members). Controlling the cell fate by efficient manipulation of ER stress mechanisms (both adaptive responses and apoptosis) is expected to create new therapeutic avenues for insulin resistance and diabetes.

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