ER stress and development of type 1 diabetes

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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 insulitis. 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 patients[12] 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

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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 IκB 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 stressmediated 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-dependent 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

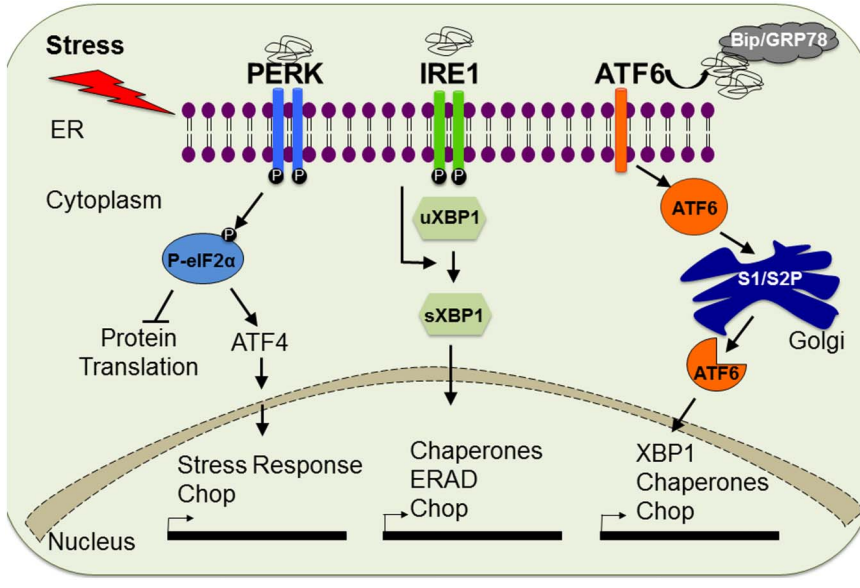

Figure 1 Unfolded protein response pathway. The UPR is triggered on ER stress and mediated by 3 sensors (PERK, IRE1/SXBP1, and ATF6) that are localized at the ER membrane.

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 roie 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) mice[44] 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 sXBPl 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 TlD. To test this hypothesis, we administered TUDCA to 2 different mouse models of TlD at the prediabetic stage and detected a striking reduction in diabetes with TUDCA treatment in these models. Histological analyses indicated a markedly decreased insulitis and immune-mediated destruction in the islet area of TUDCA-treated mice. In line with reduced insulitis, 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 TlD 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 ATF6α-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 ATF6α-deficient and control mice treated with vehicle. However, TUDCA's protective effects were completely lost in mice with β-cell-specific ATF6α deficiency, demonstrating that the effect of TUDCA in TlD 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).

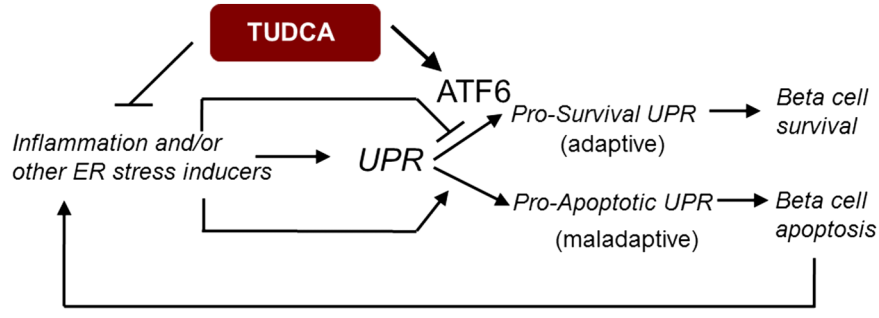

Figure 2 Tauroursodeoxycholic acid's effects in T1D. Inflammation or ER stress inducers can initiate the UPR. If ER stress is chronic and unresolvable, maladaptive UPR leads to β-cell apoptosis and death. This in turn can induce inflammation and ER stress. Tauroursodeoxycholic acid, by reducing inflammation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

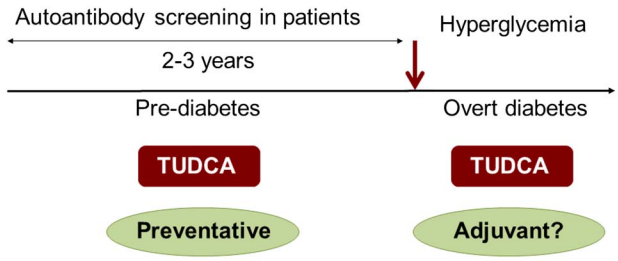

Figure 3 The translational possibilities of TUDCA. Tauroursodeoxycholic acid or other agents that could enhance ER function and restore the UPR might be used as preventative therapeutic agents for T1D. By autoantibody screening, people at high risk for T1D can be detected several years before the onset 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 organelle 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.

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