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The binary switch that controls the life and death decisions of ER stressed beta cells

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Abstract

Diabetes mellitus is a group of common metabolic disorders defined by hyperglycemia. One of the most important factors contributing to hyperglycemia is dysfunction and death of β cells. Increasing experimental, clinical, and genetic evidence indicates that endoplasmic reticulum (ER) stress plays an important role in β cell dysfunction and death during the progression of type 1 and type 2 diabetes as well as genetic forms of diabetes such as Wolfram syndrome. The mechanisms of ER stress-mediated β cell dysfunction and death are complex and not homogenous. Here we review the recent key findings on the role of ER stress and the unfolded protein response (UPR) in β cells and the mechanisms of ER stress mediated β cell dysfunction and death. Complete understanding of these mechanisms will lead to novel therapeutic modalities for diabetes.

I. Introduction

Diabetes mellitus is a group of common metabolic disorders defined by hyperglycemia. One of the most important factors contributing to hyperglycemia is dysfunction and death of β cells [1,2]. Increasing experimental, clinical, and genetic evidence indicates that endoplasmic reticulum (ER) stress plays a role in β cell dysfunction and death during the progression of type 1 and type 2 diabetes as well as genetic forms of diabetes such as Wolfram syndrome [3–6]. ER stress activates a network of signaling pathways collectively termed the Unfolded Protein Response (UPR). The UPR primarily functions to mitigate ER stress, maintain β cell function, and promote β cell survival. However; in the disease state, the UPR initiates β cell dysfunction and apoptosis. In this article, we review recent key findings on ER stress in the β cell and the potential mechanisms of ER stress-mediated β cell dysfunction and death.

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II. ER stress and the role of the Unfolded Protein Response in β cells

ER in the β cell

Pancreatic β cells produce and secrete insulin to control blood glucose levels. The ER of β cells plays a key role in the regulation of insulin production. The ER houses a specialized chemical and protein environment as well as quality control mechanisms to ensure the proper folding and processing of secretory proteins including insulin, establishing ER homeostasis. ER homeostasis is defined as the balance between the ER protein load and the ER folding capacity to handle this load. β cells often undergo conditions that stimulate insulin production and therefore a disruption to ER homeostasis. This disruption leads to the accumulation of unfolded and misfolded proinsulin in the ER lumen causing ER stress.

Three distinct functions of the UPR in β cells

ER stress is sensed by the luminal domains of three ER transmembrane proteins: Inositol Requiring 1 (IRE1), PKR-like ER kinase (PERK), and Activating Transcription Factor 6 (ATF6). These stress sensors subsequently become activated regulating a complex signaling cascade termed the unfolded protein response (UPR) [7]. The UPR regulates several downstream effectors with the following functions: adaptive response, feedback control, and cell fate regulation [3] (Figure 1). Initially the UPR triggers the adaptive response upregulating the expression of molecular chaperones and protein processing enzymes to increase folding and handling efficiency, attenuating translation and mediating mRNA degradation to reduce ER workload and prevent further accumulation of unfolded proteins, and increasing the expression of ER-associated protein degradation (ERAD) and autophagy components to promote clearance of unwanted proteins. These responses are important for β cells to mitigate ER stress and restore ER homeostasis to ensure the proper production of high quality proteins such as insulin. As ER stress is attenuated, the UPR has built in feedback control mechanisms to turn off the UPR master regulators and their downstream effectors to prevent harmful UPR hyperactivation. The UPR also regulates the expression or activation of both survival and death factors [3]. The ultimate cell fate decision determined by the UPR will be discussed in detail later.

Causes of ER stress in β cells

ER stress is triggered by a variety of stimuli and subsequently activates the UPR in a unique manner for a given cell type. There are several physiological, environmental, and genetic causes of ER stress specific for β cells (Figure 2). Under high glucose conditions, increased insulin biosynthesis overwhelms the ER folding capacity leading to ER stress. Both acute (1–3 hours) and chronic (≥ 24 hours) high glucose (≥ 16.7 mM) induces IRE1 α phosphorylation in β cells, leading to upregulation of some ER stress markers [8]. Transient activation of IRE1 α by acute high glucose enhances proinsulin biosynthesis with minimal XBP-1 splicing, whereas prolonged activation of IRE1 α by chronic high glucose leads to insulin mRNA degradation, XBP-1 splicing, and induction of other effectors leading to β cell dysfunction and death [8,9].

It has recently been shown that free fatty acids (FFAs), specifically long-chain FFAs, also induce ER stress in β cells perhaps by depleting ER calcium levels. [10,11] (Figure 2). Treatment of β cell lines with the saturated FFA, palmitate causes ER distension and activation of all three UPR stress sensors in β cell lines. The UPR may contribute to FFA-induced β cell death through CHOP upregulation and activation of JNK and caspase-12 [12].

Inflammatory cytokines, interleukin-1 β (IL-1 β) in combination with γ -interferon (IFN- γ), cause ER stress and UPR activation in β cells [13]. IL-1 β with IFN- γ has been known to induce nitric oxide (NO) in β cells, leading to β cell dysfunction and death in type 1 diabetes

[14]. It has been shown that NO-induced β cell apoptosis is mediated by ER stress [15]. NO production decreases expression of the sarcoendoplasmic reticulum pump Ca^{2+} ATPase 2b (SERCA2b), leading to a decrease in Ca^{2+} in the ER. This Ca^{2+} depletion causes a high level of ER stress in β cells [13,16]. This ER stress may have a role in cytokine induced-cell death in Type 1 diabetes.

Proinsulin misfolding causes ER stress in β cells. The Akita diabetes mouse model carries a cysteine 96 to tyrosine substitution in the insulin 2 gene [17]. This mutation disrupts a disulfide bond of mature insulin causing incorrect folding of proinsulin in the ER, leading to ER stress and subsequent death of β cells [18,19]. It has recently been shown that mutations in the human insulin gene, specifically mutations occurring in critical regions of proinsulin folding can also cause this kind of pathology [20]. This is an autosomal dominant disorder, leading to permanent neonatal diabetes.

Islet amyloid polypeptide (IAPP) is secreted along with insulin in β cells. Islet amyloid, composed of a 37-residue amyloidogenic polypeptide derived from IAPP, is commonly found in the pancreatic islets of Type 2 diabetes patients and may play a role in β cell dysfunction and death during the progression of the disease [21]. IAPP spontaneously forms ER membrane-damaging sheets of amyloid [22]. It has been shown that high expression of human IAPP induces ER stress and subsequent death of β cells [23,24]. Therefore, IAPP could be a link between ER stress and β cell death in type 2 diabetes.

Tolerable and Unresolvable ER stress in β cells

We propose that there are two types of ER stress conditions: tolerable and unresolvable. Under tolerable ER stress conditions, the UPR promotes β cell adaptation and survival. In contrast, under unresolvable ER stress conditions, the UPR induces β cell death (Figure 3A).

β cells are exposed to physiological conditions that induce mild ER stress such as transient high glucose. These ER stress conditions are tolerable and the UPR can restore ER homeostasis, leveraging physiological functions and promoting cell survival. Therefore the UPR has a very important role in β cell physiology (Figure 3B).

Under unresolvable conditions in which the UPR fails to attenuate ER stress and restore ER homeostasis, the UPR induces β cell dysfunction and apoptosis. This unresolvable ER stress can be caused by genetic mutations such as insulin gene mutations as well as environmental factors such as inflammatory cytokines, free fatty acids and chronic high glucose (Figure 3C).

III. The UPR and the regulation of β cell fate under tolerable and unresolvable ER stress conditions

UPR as a binary switch regulating the life and death of β cells

The UPR determines β cell fate by behaving like a binary switch between life and death. The outcome of this switch depends on the nature of the ER stress condition, whether it is tolerable or unresolvable, the activation and regulation of the UPR stress sensors, and the balance of UPR regulated death and survival components (Figure 4). The mechanisms of this switch are not completely understood but recent findings have shed some clues. It is important to emphasize that the β cell UPR is unique and tailored to the needs of the β cell. Here we discuss the UPR signaling network and its regulation of cell fate under tolerable and unresolvable ER stress conditions in the context of the β cell.

Regulation of Survival and Death effectors by the UPR master regulators

IRE1, PERK and ATF6 regulate both survival/adaptive and death effectors. Some of these effectors are simultaneously expressed and the balance between the components determines life and death. Other effectors are specifically turned on or upregulated depending on the ER stress conditions.

IRE1 is the most characterized master regulator of the UPR. Its baseline expression is higher in β cells compared to other cell types [8]. In response to tolerable ER stress conditions, IRE1 autophosphorylates and activates its RNase domain. This activated RNase domain cleaves X-box binding protein 1 (XBP1) mRNA into an active transcription factor. [25–27]. Active XBP-1 regulates expression levels of homeostatic components of the UPR such as molecular chaperones and ERAD enzymes [28,29]. IRE1 also cleaves ER associated mRNAs to presumably reduce ER workload. Furthermore IRE1 has a key role in insulin biosynthesis. Under acute high glucose conditions, IRE1 is activated and enhances proinsulin biosynthesis, while disruption of IRE1 signaling leads to a suppression of proinsulin biosynthesis. Thus, IRE1 activation has a role in β cell function and promotes adaptation to physiological ER stress conditions.

In contrast, under unresolvable conditions, IRE1 becomes hyperactivated and as a result have several harmful outputs. IRE1 hyperactivation directly activates apoptotic pathways. In the presence of unresolvable ER stress, IRE1 recruits TRAF2 and phosphorylates ASK1. Activated ASK1 phosphorylates JNK which regulates BCL2 family members to induce apoptosis. [30–33]. Prolonged IRE1 activation also induces the decay of mRNAs encoding ER homeostatic proteins, including PDI, and BiP, therefore decreasing the ER folding capacity promoting apoptosis [9,34–36].

PERK was discovered as the second master regulator of the UPR [37]. Expression level of PERK is higher in β cells than in other cells. PERK has a role in β cell function under physiological ER stress conditions. PERK has been shown to regulate β cell proliferation and development [38,39]. Tissue- and cell-specific knockouts of the PERK gene in mice cause severe defects in fetal and neonatal β cell proliferation and differentiation, leading to low β cell mass, defects in proinsulin trafficking and insulin secretion, and diabetes [38,39]. PERK also plays a role in producing proper amount of proinsulin in response to hyperglycemia. In PERK deficient islets proinsulin production is markedly increased in response to transient high glucose as compared to control islets [40]. This unregulated production of proinsulin leads to β cell death and subsequent diabetes.

PERK has been shown to protect β cells from ER stress-mediated cell death [40,41]. Under tolerable ER stress conditions, activated PERK phosphorylates eIF2 α which leads to attenuation of global mRNA translation reducing ER protein work load and promoting adaption. Meanwhile phosphorylated eIF2 α favors the translation of selected mRNAs such as activating transcription factor 4 (ATF4) [42]. ATF4 regulates genes involved in restoring ER homeostasis as well as genes involved in antioxidative stress response and amino acid biosynthesis. We have recently found that PERK upregulates a novel anti-apoptotic effector, apoptosis antagonizing transcription factor (AATF) and mediates β cell survival in part through the transcriptional regulation of AKT1 [43]. Under unresolvable ER stress conditions, continuous eIF2 α phosphorylation induces β cell death [11]. In these conditions, ATF4 induces the expression of the pro-apoptotic transcription factor CHOP [15,42,44–47]. CHOP regulates the expression of BCL-2 family members as well as other apoptotic components promoting cell death.

ATF6 α is the third master regulator of the UPR. Upon ER stress conditions, ATF6 α transits to the golgi apparatus and is cleaved into an active transcription factor [48]. This processed

form of ATF6 α translocates to the nucleus to increase expression of homeostatic effectors involved in protein folding, processing, and degradation [49,50]. However, hyperactivation of ATF6 α leads to β cell dysfunction and death. The effectors responsible for this phenotype are currently unknown[51]. In addition, it has been shown that ATF6 α hyperactivation suppresses insulin gene expression. Under unresolvable ER stress, ATF6 is activated, leading to a decrease in insulin gene expression [51].

The apoptotic and survival components of the UPR are also regulated at the post-transcriptional level. It has been shown that survival is favored during mild and tolerable ER stress as a consequence of the intrinsic instabilities of mRNAs and proteins that promote apoptosis such as CHOP compared to those that facilitate protein folding and adaptation such as BiP. As a consequence, the expression of apoptotic proteins is short-lived as cells adapt to stress [52]. This observation indicates that post-transcriptional mechanisms regulating the ratio between survival and apoptotic effectors are important for controlling life and death of β cells.

Regulation of the UPR master regulators

As we discussed so far, the UPR regulates both survival and death effectors. The ultimate cell fate consequence regulated by these effectors also depends on the tight regulation of the UPR master regulators. Under tolerable ER stress conditions, the UPR master regulators and downstream components are properly turned off as ER homeostasis is reestablished. However under unresolvable ER stress conditions, the UPR master regulators bypass this feedback regulation, causing harmful hyperactivation, which favors the activation of apoptotic pathways and the upregulation of death effectors.

Some of the feedback effectors involved in the regulation of the UPR activation include BiP, Gadd34, P58^{IPK}, RACK1 and WFS1. Under normal conditions, BiP binds to the UPR master regulators and prevents their premature activation [53–55]. Under ER stress conditions, BiP binds to accumulating unfolded proteins leading to the activation of the UPR master regulator. Gadd34 dephosphorylates eIF2 α by recruiting phosphatase 1 (PP1) in order to restore protein translation as ER homeostasis is achieved. GADD34 is induced by ER stress under the PERK-eIF2 α -ATF4 pathway [56]. P58^{IPK} is another negative regulator of PERK signaling and functions in maintaining ER homeostasis in β cells. P58^{IPK} expression is induced by ER stress mediated by ATF6 and is highly expressed in the pancreas. It has been shown that P58^{IPK} interacts with the kinase domain of PERK and inhibits its activity [57]. P58^{IPK} knockout mice show a gradual onset of glucosuria and hyperglycemia associated with increased apoptosis of islet cells [58]. Recently it has been discovered that IRE1 α activation is tightly regulated by the adaptor protein RACK1. Under physiological ER stress conditions such as acute glucose, RACK1 binds to IRE1 α and recruits protein phosphatase 2A (PP2A) to turn off IRE1 activation, preventing its hyperactivation [59].

We have recently discovered that WFS1, a causative gene for Wolfram syndrome, is a key negative regulator of the UPR [5]. Wolfram syndrome is a rare autosomal recessive disorder characterized by childhood onset of diabetes mellitus, followed by optic atrophy, deafness and death from neurodegeneration in the third or fourth decades [60–62]. Postmortem studies reveal a non-autoimmune-linked selective loss of β cells [63]. Mutations in the WFS1 gene causes Wolfram syndrome [64–71]. WFS1 is localized at the ER and has a protective function against ER stress; however, the mechanisms were unclear. Our group found that the WFS1 gene plays an important role in regulating ATF6 α activation [5]. In healthy cells, WFS1 prevents activation of ATF6 signaling by recruiting ATF6 α to HRD1 and the proteasome for ubiquitin-mediated degradation under non-ER stress conditions. Under ER stress conditions, ATF6 α is released from WFS1 and is free to regulate stress

signaling targets in the nucleus. As ER homeostasis is reestablished, WFS1 is induced by ER stress, which would cause the eventual degradation of ATF6 α . In patients with Wolfram syndrome, ATF6 α constantly escapes from WFS1-mediated degradation and is hyperactivated regardless of the ER stress conditions. This ATF6 α hyperactivation induces β cell death. Recent genome-wide associate studies have suggested that dysfunctional WFS1 may also have a role in the progression of type 2 diabetes [72–74].

Taken together, under tolerable ER stress conditions the UPR is properly turned off favoring β cell survival. However; under unresolvable ER stress conditions, the UPR master regulators are hyperactivated bypassing negative regulation therefore tipping the balance towards β cell apoptosis.

IV. Concluding remarks

Increasing clinical, experimental, and genetic evidence indicates that ER stress and the UPR has a role in β cell dysfunction and death during the progression of type 1, type 2 and genetic forms of diabetes. It is currently believed that the UPR regulates β cell fate by behaving as a binary switch between life and death. The complete understanding of this UPR switch will provide us new insights into the mechanisms of β cell death during diabetes and shed light on future diabetes prevention or treatment.

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Figure 1. Homeostatic, Feedback, and Cell Fate Regulation by the UPR

ER stress is sensed by three master regulators of the UPR: IRE1, PERK, and ATF6. These master regulators subsequently become activated, regulating a complex signaling cascade termed the unfolded protein response (UPR). The UPR regulates several downstream effectors with three distinct functions: adaptive response, feedback control, and cell fate regulation.

Figure 2. β cell specific inducers of ER stress

There are several physiological, environmental, and genetic causes of ER stress specific for β cells. High glucose conditions cause an increase in proinsulin ER load. Free fatty acids and cytokines decrease ER calcium levels inhibiting the functions of ER chaperones and folding enzymes. Human IAPP spontaneously forms ER membrane-damaging sheets of amyloid. Mutant insulin misfolds and accumulates in the ER.

Figure 3. Tolerable and Unresolvable ER stress in β cells

- A.** There are two types of ER stress conditions: tolerable and unresolvable. Tolerable ER stress can be physiological, acute, and/or mild. Under tolerable ER stress conditions, the UPR is properly activated and regulated reducing ER stress and promoting β cell adaptation and survival. In contrast, unresolvable ER stress conditions are pathological, chronic, and/or severe. Under unresolvable ER stress conditions, the UPR is hyperactivated inducing β cell dysfunction and death.
- B.** When β cells are exposed to physiological stimuli that induces tolerable ER stress, the UPR triggers transcription of adaptive and survival genes, attenuates translation, and promotes mild mRNA degradation to reduce ER stress. The UPR also promotes proinsulin biosynthesis. As ER homeostasis is reestablished, BiP, Gadd34, P58^{IPK}, RACK1 and WFS1 properly turn off different components of the UPR.
- C.** When β cells are under pathological conditions that induce unresolvable ER stress, the UPR is hyperactivated triggering the activation of apoptotic pathways and upregulating apoptotic genes. The UPR also mediates degradation of mRNAs encoding proinsulin and ER homeostatic proteins. Under these conditions, the UPR bypasses feedback regulation.

Figure 4. The UPR determines β cell fate by behaving like a binary switch

The UPR determines β cell fate by behaving like a binary switch between life and death. The outcome of this switch depends on the nature of the ER stress conditions, the activation and regulation of the UPR stress sensors, and the balance of UPR regulated death and survival components.