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A link between endoplasmic reticulum stress-induced β-cell apoptosis and the group VIA Ca^{2+}-independent phospholipase A_2 ($iPLA_2\beta$)

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Abstract

Endoplasmic reticulum (ER) stress is becoming recognized as an important contributing factor in various diseases, including diabetes mellitus. Prolonged ER stress can cause β-cell apoptosis; however, the underlying mechanism(s) that contribute to this process are not well understood. Early reports suggested that arachidonic acid metabolites and a Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) activity play a role in β-cell apoptosis. The PLA_2 family of enzymes catalyse the hydrolysis of the *sn*-2 substituent (i.e. arachidonic acid) of membrane phospholipids. In light of our findings that the pancreatic islet β-cells are enriched in arachidonate-containing phospholipids and express the group VIA $iPLA_2\beta$, we considered the possibility that $iPLA_2\beta$ participates in ER stress-induced β-cell apoptosis. Our work revealed a novel mechanism, involving ceramide generation and triggering of mitochondrial abnormalities, by which $iPLA_2\beta$ participates in the β-cell apoptosis process. Here, we review our evidence linking ER stress, β-cell apoptosis and $iPLA_2\beta$. Continued studies in this area will increase our understanding of the contribution of $iPLA_2\beta$ to the evolution of diabetes mellitus and will further our knowledge of factors that influence β-cell health in diabetes mellitus and identify potential targets for future therapeutic interventions to prevent β-cell death.

Keywords

apoptosis; β-cell; ceramides; $iPLA_2\beta$; mitochondria

Introduction

Diabetes mellitus is the most prevalent human metabolic disease, and it results from loss and/or dysfunction of β-cells in pancreatic islets. Type 2 diabetes mellitus (T2DM) results from a progressive decline of β-cell function and chronic insulin resistance [1]. Autopsy studies indicate that the β-cell mass in obese T2DM patients is smaller than that in obese non-diabetic subjects [2–4] and that the loss in β-cell function in non-obese T2DM is associated with decreases in β-cell mass [5,6]. β-Cell mass is regulated by a balance

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Conflict of Interests

The authors do not declare any conflict of interest relevant to this manuscript.

between β-cell growth, resulting from β-cell replication and neogenesis, and β-cell death resulting from apoptosis [7–9]. Findings in both rodent models of T2DM [10] and human T2DM [6] and other observations [11–13] suggest that the decrease in β-cell mass in T2DM is not because of reduced β-cell proliferation or neogenesis but may be a consequence of increased β-cell apoptosis. Emerging evidence suggests that cytokine-mediated β-cell apoptosis is also a prominent contributor to β-cell death during the development of autoimmune type 1 diabetes mellitus (T1DM) [14–17]. It is therefore important to understand the mechanisms underlying β-cell apoptosis if this process is to be prevented or delayed.

Apoptosis can be mediated via an extrinsic (death receptor) or intrinsic (mitochondrial) pathway [18,19]. Recently, apoptosis as a result of prolonged endoplasmic reticulum (ER) stress has gained recognition [18,20–22] and this process has been implicated as a causative factor in Alzheimer's and Parkinson's diseases and cancer [23]. Several studies suggest that ER stress can also cause β-cell apoptosis, a consequence of which is diabetes mellitus. For instance, β-cell death in the Akita [24,25] and NOD.k iHEL non-immune [26] diabetic mouse models is reported to be due to ER stress and mutations in genes encoding the ER-stress transducer pancreatic ER kinase (PERK) [27], and the ER-resident proteins involved in the degradation of malfolded ER proteins have been linked to diminished β-cell health clinically [28,29]. Additionally, ER stress is thought to play a role in the autoimmune destruction of β-cells during the development of T1DM [17,30–34].

The ER, in addition to serving as a cellular Ca^{2+} store, is the site where secretory proteins are synthesized, assembled, folded and posttranslationally modified. Interruption of any of these functions can lead to production of malfolded proteins and their accumulation in the ER. When an imbalance between the load of client proteins on the ER and the ER's ability to process the load occurs, it results in ER stress [35,36]. Prolonged ER stress promotes induction of stress factors and activation of caspase-12, localizedintheER [19,21,37], andcansubsequently lead to downstream activation of caspase-3, a protease that is central to the execution of apoptosis [38]. Being a site for Ca^{2+} storage, the ER responds to various stimuli to release Ca^{2+} and is therefore extremely sensitive to changes in cellular homeostasis. Thapsigargin, which depletes ER Ca^{2+} stores by inhibiting sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) Ca^{2+} pumps, causes ER stress in pancreatic islets and promotes hydrolysis of arachidonic acid. We found that the accumulation in arachidonic acid is suppressed by a bromoenol lactone (BEL) suicide substrate inhibitor of the group VI Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) [39]. These observations raised the possibility that $iPLA_2\beta$ is activated during ER stress in β-cells.

The PLA_2s are a diverse group of enzymes that catalyse the hydrolysis of the *sn*-2 substituent from glycerophospholipid substrates to yield a free fatty acid and a 2-lysophospholipid [40]. In pancreatic islet β-cell organelles, similar to brain tissue [41], arachidonic acid is a major *sn*-2 substituent of membrane phospholipids [42,43]. Arachidonic acid and its oxygenated metabolites are potent bioactive mediators that can regulate physiological and pathophysiological processes. At present, the recognized PLA_2s are classified into 15 groups based on their Ca^{2+} requirement for activation and sequence homology [44,45]. Among the $iPLA_2$s, the β-isoform of $iPLA_2$ ($iPLA_2\beta$) does not require Ca^{2+} for activity [45]. The $iPLA_2\beta$ enzyme is activated by ATP, inhibited by a BEL suicide substrate [46], predominantly cytosolic under basal conditions, and has unique features that enhance the possibility that it can participate in multiple biological processes [45,47]. These include ankyrin repeats, acyl-CoA esterase activity, caspase-3 cleavage consensus sequence, bipartite nuclear localization sequence and calmodulin-binding domain. In addition, the $iPLA_2\beta$ gene promoter contains a sterol regulatory element (SRE), and transcription of the $iPLA_2\beta$ gene is regulated in a sterol-dependent manner [48].

The iPLA$_2$β has been proposed to be involved in phospholipid remodelling, maintenance of phosphatidylcholine mass, signal transduction, cell proliferation and other biological processes [45]. Furthermore, a number of studies have linked iPLA$_2$β with apoptosis in non-β-cells. Our studies revealed that in pancreatic islets iPLA$_2$β is expressed predominantly in the β-cells and that it, as expected, is activated by ATP and inhibited by BEL [46,49–51]. In view of subsequent demonstrations that inhibition of SERCA induces apoptosis through a Ca^{2+}-independent mechanism [52] and that iPLA$_2$β over-expression induces U937 cell apoptosis [53], we considered the possibility that iPLA$_2$β participates in ER stress-induced β-cell apoptosis. Here, we review our observations over the past 5 years that provide evidence for the involvement of iPLA$_2$β in ER stress-induced β-cell apoptosis via a previously unrecognized mechanism of ceramide generation in the β-cell.

iPLA$_2$β Role in β-Cells Undergoing ER Stress

Link Between ER Stress-induced β-Cell Apoptosis and iPLA$_2$β

A potential involvement of iPLA$_2$β in β-cell apoptosis was suggested by studies from Polonsky [52] and Kudo [53]. The first study showed that thapsigargin and other SERCA inhibitors induce MIN-6 cell apoptosis by a mechanism that did not require an increase in $[Ca^{2+}]_i$ but one that involved generation of arachidonic acid metabolites. This implied involvement of a PLA$_2$ that manifested activity in the absence of Ca^{2+}. In the latter study, overexpression of iPLA$_2$β, but not cPLA$_2$, increased the incidence of human monocytic U397 cells exposed to TNFα/cycloheximide. Interestingly, cells undergoing apoptosis expressed both the expected full-length iPLA$_2$β and an additional immunoreactive band of smaller size. This band was deduced to be a truncated iPLA$_2$β generated by caspase-3-mediated cleavage of the full-length iPLA$_2$β. Surprisingly, the truncated iPLA$_2$β was found to manifest greater activity and overexpression of this protein induced more pronounced apoptosis than the full-length iPLA$_2$β. These findings suggested that activation of iPLA$_2$β is part of the mechanism that contributes to apoptotic cell death. These reports caused us to consider whether iPLA$_2$β participates in ER stress-induced β-cell apoptosis. To address this possibility, we characterized the effects of inducing ER stress in rat INS-1 cells [54,55], which express an iPLA$_2$β activity [51].

Exposure of INS-1 cells to thapsigargin promoted time-dependent increases in ER stress and apoptotic factors (Grp78/Bip, pPERK, p-eIF2a, CHOP and activated caspase-12). Prolonged ER stress led to the activation of caspase-3, cleavage of PARP and INS-1 cell apoptosis. Inhibition of iPLA$_2$β, however, suppressed, whereas overexpression of iPLA$_2$β amplified the ER stress-induced INS-1 cell apoptosis. These observations are consistent with the participation of iPLA$_2$β in ER stress-induced β-cell apoptosis.

To eliminate the possibility that the above-described observations in INS-1 cells were as a result of non-specific effects of chemical inhibitors or a consequence of iPLA$_2$β overexpression, we examined whether iPLA$_2$β plays a role in the spontaneous development of ER stress. For these studies, we used a β-cell line (*Ins2*$^{+/AK}$) established from Akita mice [56] which harbours a spontaneous mutation of the insulin 2 gene (Ins2) (C96Y). This mutation causes misfolding of insulin in the ER leads to the development of hyperglycaemia/diabetes as a consequence of ER stress-induced β-cell apoptosis [57,58]. Akita β-cells express higher pPERK and activated caspase-3 and undergo a higher incidence of apoptosis under basal conditions, in comparison with wild-type β-cells [59]. As expected, ER stress accelerated and/or amplified these outcomes in the Akita β-cells, relative to wild-type β-cells. Consistent with our observations in INS-1 cells, iPLA$_2$β expression was greater in the Akita β-cells than in the wild-type β-cells. Importantly, both basal and ER stress-associated apoptosis were suppressed in Akita cells treated with iPLA$_2$β-targeted siRNAs.

These findings confirm our hypothesis that iPLA$_2$β expression/activation is a critical step in ER stress-mediated β-cell apoptosis. The physiological relevance of these observations in cell lines is underscored by our most recent work (*manuscripts in preparation*) in islets from iPLA$_2$β-null, islet β-cell-specific iPLA$_2$β transgenic and NOD mice as well as in native human pancreatic islets. Furthermore, these observations are the first to identify regulation of iPLA$_2$β expression during the progression of a biological process.

Link Between ER Stress-induced Ceramide Generation and iPLA$_2$β

Ceramides are lipid messengers that can suppress cell growth and induce apoptosis [60] and they can be generated via multiple mechanisms (*de novo* synthesis, sphingomyelin hydrolysis, inhibition of ceramide degradation or salvage pathway). While examining lipid profiles that might be associated with ER stress, we unexpectedly found a temporal increase in ceramides in the INS-1 cells [61]. Intriguingly, examination of the activated pathway revealed that ER stress promotes ceramide generation by inducing neutral sphingomyelinase (NSMase) [55] and that inactivation of iPLA$_2$β by BEL not only suppresses ceramide accumulation but also inhibits NSMase induction. These findings suggested that the ceramide generation during ER stress in β-cells occurs by an iPLA$_2$β-dependent induction of NSMase. Consistent with these findings, overexpression of iPLA$_2$β amplified NSMase induction, sphingomyelin hydrolysis and ceramide generation, whereas inhibition or knockdown of NSMase suppressed sphingomyelin hydrolysis, ceramide generation, DNA laddering and TUNEL positivity in INS-1 cells. Taken together with the evidences of increased basal expression of NSMase and lower total pool of sphingomyelin pools in the Akita, relative to wild-type, β-cells and inhibition of ER stress-associated NSMase induction in both cells by BEL, our findings reveal that the iPLA$_2$β/ceramide axis plays an important role during ER stress-induced β-cell apoptosis.

Link Between ER Stress, Mitochondrial Apoptotic Pathway and iPLA$_2$β

Ceramide accumulation has been linked to apoptosis through the intrinsic (mitochondrial) pathway. Although the ER- and mitochondria-associated apoptotic pathways can be activated independently, it has been suggested that induction of ER stress may lead to the triggering of intrinsic apoptotic processes [62,63]. Our findings suggest that such a scenario evolves in β-cells undergoing ER stress as cytochrome *c* and Smac accumulate in the cytosol of thapsigargin-treated INS-1 cells and this response is magnified in cells that overexpress iPLA$_2$β [55]. These findings suggested the unexpected involvement of iPLA$_2$β in the mitochondrial triggering process following induction of ER stress. Because the release of cytochrome *c* is associated with alterations in the mitochondrial membrane integrity, assessment of mitochondrial membrane potential ($\Delta\psi$) and permeability transition pore (PTP) revealed a loss in $\Delta\psi$ and activation (opening) of the PTP in ER-stressed cells. These outcomes were amplified in iPLA$_2$β-overexpressing cells and suppressed by the inactivation of iPLA$_2$β by BEL. Basal $\Delta\psi$ was also compromised in the Akita β-cells, in comparison with the wild-type β-cells. Furthermore, inhibition or knockdown of NSMase suppressed the loss in $\Delta\psi$ and activation of PTP. These findings therefore indicate that the iPLA$_2$β – ceramide axis plays a critical role in activating the mitochondrial apoptotic pathway in insulin-secreting cells during ER stress.

Regulation of iPLA$_2$β During ER Stress

To date, our findings suggest that there are at least three mechanisms that can potentially regulate iPLA$_2$β activity in β-cells during ER stress: translocation of iPLA$_2$β to subcellular organelles, proteolytic processing of iPLA$_2$β to a truncated, more highly active form and increases in iPLA$_2$β expression. Like cPLA$_2$, iPLA$_2$β translocates from cytosol to various subcellular organelles on exposure to certain stimuli [64]. Earlier, we observed that ER stress promotes the accumulation of iPLA$_2$β in the perinuclear region [61]. Subsequent

studies revealed that following induction of ER stress, iPLA$_2$β accumulates first in the ER [54] and then in the mitochondria [55]. In parallel, enzymatic activity assays revealed increases in nuclear-, ER- and mitochondria-associated iPLA$_2$β catalytic activity. The increase in mitochondria-associated iPLA$_2$β is accompanied by a concurrent decrease in ER iPLA$_2$β, suggesting that iPLA$_2$β translocates from the ER to the mitochondria during the evolution of ER stress [65]. Consistent with such iPLA$_2$β mobilization, sphingomyelin hydrolysis and ceramide accumulation were increased in the ER and mitochondria fractions of INS-1 cells undergoing ER stress and both outcomes were twofold greater in iPLA$_2$β-overexpressing INS-1 cells [55]. This is an intriguing scenario because pancreatic islet β-cell organelles are enriched in arachidonic acid-containing membrane phospholipids [42,43] and arachidonic acid and arachidonoyl-CoA can activate NSMase [66]. Thus, in addition to inducing NSMase expression, products derived from iPLA$_2$β activation can stimulate NSMase activity. Our findings therefore suggest that ER stress promotes association of iPLA$_2$β with subcellular organelles (nucleus, ER and mitochondria) where it generates bioactive lipids that are recognized contributors to the apoptotic process.

Curiously, the iPLA$_2$β that accumulates in the nucleus is predominantly a truncatedprotein [61]. ThistruncatediPLA$_2$β isoform is analogous to the one previously reported to be a product of caspase-3-catalysed cleavage at the caspase-3 consensus site of iPLA$_2$β in the N-terminal region [53]. Caspase-3 inhibition prevents generation of the truncated iPLA$_2$β product. Mobilization of the truncated iPLA$_2$β, described as being more active than the full-length iPLA$_2$β [53] in the nucleus, supports the possibility that the shorter iPLA$_2$β itself or products derived from iPLA$_2$β-mediated hydrolysis of nuclear membrane phospholipids could regulate transcription of genes, such as the one encoding NSMase.

ER stress induces iPLA$_2$β expression in both wild-type and Akita β-cell [59], suggesting that a pathway that promotes iPLA$_2$β expression is activated during ER stress in the β-cells. One report identified the presence of a SRE in the iPLA$_2$β gene [48]. Because SRE-binding proteins (SREBPs) are known to be induced and processed to the mature (active) forms (mSREBPs) during stress and also in response to glucolipotoxicity and thapsigargin in β-cells [67], we considered the possibility that the increase in iPLA$_2$β expression observed in our studies was induced by elevations in mSREBPs. Indeed, expression of mSREBP-1 was higher in the Akita than in the wild-type β-cells and increased in response to ER stress. A dominant-negative SREBP-1 [68] suppressed both ER stress-induced processing of SREBP-1 and expression of iPLA$_2$β [59], consistent with an SREBP-mediated regulation of iPLA$_2$β expression in β-cells undergoing ER stress.

Summary and Conclusions

β-Cell apoptosis as a consequence of prolonged ER stress is becoming recognized as a contributing factor in the development and progression of diabetes mellitus. Our observations reveal a role for iPLA$_2$β-mediated ceramide generation via NSMase during ER stress-induced β-cell apoptosis, as summarized in figure 1. Evidence gathered in cell lines, native pancreatic islets and diabetic animal models indicates participation of the iPLA$_2$β in this process. To date, we have determined that ER stress promotes activation of iPLA$_2$β, accumulation of iPLA$_2$β in subcellular organelles that are integral to the apoptotic process, proteolytic cleavage of iPLA$_2$β to a more active product, ceramide generation by an iPLA$_2$β-dependent mechanism and mitochondrial abnormalities by an iPLA$_2$β/ceramide-dependent mechanism, and that ER stress-induced β-cell apoptosis is amplified by iPLA$_2$β overexpression and suppressed by the inactivation of iPLA$_2$β. Also recognized was a regulation of iPLA$_2$β expression during a physiological response by mSREBP-1. Furthermore, β-cells from a spontaneous model of ER stress express higher iPLA$_2$β and NSMase and exhibit a profile similar to that evidenced in INS-1 cells exposed to SERCA

inhibitors, strengthening a link between ER stress, β-cell apoptosis and iPLA$_2$β. Finally, evidence of higher iPLA$_2$β and NSMase expression in native pancreatic islets from the Akita and NOD (a model of autoimmune T1DM) mice supports the possibility that activation of these enzymes contributes to β-cell apoptosis and the eventual onset and development of diabetes mellitus in these animals.

Ongoing work in our laboratory is focused on identifying specific targets of iPLA$_2$β and products of its activation that play a role in the cellular events that lead to β-cell apoptosis. Among the emerging paradigms in these studies is the concept that β-cell apoptosis can be, in part, attributed to iPLA$_2$β-dependent modification of the unfolded protein response or triggering alternative splicing of the pre-mRNAs encoding pro-apoptotic factors. These and other studies will further our understanding of the role of iPLA$_2$β in β-cell apoptosis and enable us to more precisely define its contribution to the onset and progression of diabetes. The findings will add to our knowledge of factors that influence β-cell health in diabetes mellitus and identify potential targets for future therapeutic interventions to prevent β-cell death.

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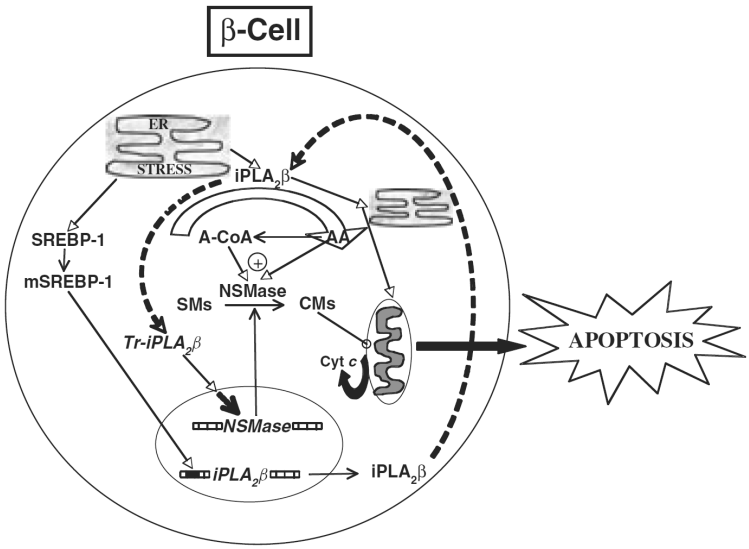

Figure 1.

Proposed role of the cytosolic group VIA Ca^{2+}-independent phospholipase A_2 ($iPLA_2\beta$)–ceramide axis in endoplasmic reticulum (ER) stress-induced β-cell apoptosis. ER stress in the β-cell promotes the expression/activity of $iPLA_2\beta$, leading to the induction of NSMase and increased hydrolysis of sphingomyelins from β-subcellular organelles and accumulation of ceramides in the cytosol. These lipid second messengers activate the mitochondrial apoptotic processes and cause loss in $\Delta\Psi$, activation of permeability transition pore and release of cytochrome *c* into the cytosol. The latter interacts with other factors to activate caspases leading to apoptosis of the β-cell. Consistent with a role for $iPLA_2\beta$-mediated ceramide generation via NSMase in this process, inactivation of $iPLA_2\beta$ or chemical inhibition or knockdown of NSMase suppresses NSMase expression and ceramide generation, cytochrome *c* release and apoptosis [54]. The broken arrows linking $iPLA_2\beta$ and truncated $iPLA_2\beta$ (Tr-$iPLA_2\beta$) indicate a series of events leading to the generation of a caspase-3-processed highly active $iPLA_2\beta$.