Original Paper

Inhibition of Prenylation Promotes Caspase 3 Activation, Lamin B Degradation and Loss in Metabolic Cell Viability in Pancreatic β -Cells

Khadija G. Syeda Anjaneyulu Kowluru

β -Cell Biochemistry Laboratory, John D. Dingell VA Medical Center, and Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI USA

Key WordsLamins • Prenylation • Caspase 3 • Statins • Pancreatic β -cells**Abstract**

Background/Aims: Lamins are intermediate filament proteins that constitute the main components of the lamina underlying the inner-nuclear membrane and serve to organize chromatin. Lamins (e.g., lamin B) undergo posttranslational modifications (e.g., isoprenylation) at their C-terminal cysteine residues. Such modifications are thought to render optimal association of lamins with the nuclear envelop. Using human islets, rodent islets, and INS-1 832/13 cells, we recently reported significant metabolic defects under glucotoxic and endoplasmic reticulum (ER) stress conditions, including caspase 3 activation and lamin B degradation. The current study is aimed at further understanding the regulatory roles of protein prenylation in the induction of the aforementioned metabolic defects. **Methods:** Subcellular phase partitioning assay was done using Triton X-114. Cell morphology and metabolic cell viability assays were carried out using standard methodologies. **Results:** We report that exposure of pancreatic β -cells to Simvastatin, an inhibitor of mevalonic acid (MVA) biosynthesis, and its downstream isoprenoid derivatives, or FTI-277, an inhibitor of farnesyltransferase that mediates farnesylation of lamins, leads to activation of caspase 3 and lamin B degradation. Furthermore, Simvastatin-treatment increased activation of p38MAPK (a stress kinase) and inhibited ERK1/2 (regulator of cell proliferation). Inhibition of farnesylation also resulted in the release of degraded lamin B into the cytosolic fraction and promoted loss in metabolic cell viability. **Conclusion:** Based on these findings we conclude that protein prenylation plays key roles in islet β -cell function. These findings affirm further support to the hypothesis that defects in prenylation pathway induce caspase-3 activation and nuclear lamin degradation in pancreatic β -cells under the duress of metabolic stress (e.g., glucotoxicity).

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Introduction

Nuclear lamins (A, B and C) surround the nucleoplasmic contents in a mesh-like network often referred to as the nuclear lamina. These intermediate filaments provide a structural framework to the nuclear envelope, play a role in arrangement of the chromatin within the nucleus, in DNA replication and also participate in DNA damage repair [1-5]. In order for lamins to be involved in these important nuclear processes, and to be functionally active, they undergo a series of post-translational modifications (farnesylation, endoproteolytic cleavage, carboxymethylation etc.; refs [6-8]). Aberrant farnesylation of lamins, especially lamin A, leads to a number of diseases affecting the striated muscle (e.g. Emery- Dreifuss muscular dystrophy, dilated cardiomyopathy), adipose tissue (e.g. Dunnigan-type familial partial lipodystrophy). Altered prenylation could also result in abnormal senescence and growth deformities (e.g. Progeria syndrome); these are referred to as laminopathies [7, 9-11]. Despite the existing evidence on functions of lamins and diseases associated with abnormal lamin processing, significant knowledge gaps still exist with regard abnormalities in lamin processing, including post-translational modifications, in pancreatic islet β -cells under conditions of glucolipotoxicity and diabetes.

Along these lines, we have recently begun to investigate potential structural abnormalities lamins in human islets, rodent islets and INS-1 832/13 cells following long-term exposure to glucotoxic conditions. We noted marked degradation of lamin B in pancreatic β -cells under these conditions [7, 12-14]. More importantly, lamin degradation is mediated *via* caspase 3 activation since specific inhibitors of caspase 3 significantly attenuated lamin B degradation under these conditions [14]. From a mechanistic standpoint, we reported recently that nifedipine, a known blocker of calcium channels, suppressed caspase 3 activation and lamin degradation under the duress of glucotoxic conditions. Lastly, 4-phenylbutyrate, an inhibitor of endoplasmic reticulum (ER) stress, also attenuated high glucose-induced caspase 3 activation and lamin B degradation [14]. Based on these observations, we concluded that high glucose-induced calcium overload and ER stress might underlie mitochondrial and nuclear dysregulation leading to loss in metabolic viability in pancreatic β -cells [7, 12-14].

As stated above, lamins undergo post-translational prenylation (e.g., farnesylation) at their C-terminal cysteine residues (Fig. 1). Farnesylation, which constitutes incorporation of 15-C farnesyl derivative of MVA, is the first committed step for subsequent modification steps including proteolytic cleavage of the three amino acid residues by protease of microsomal origin and methylation of the farnesylated cysteine. Earlier studies from our laboratory have identified localization of ZMPSTE24, a zinc metallopeptidase involved in the processing of prelamin A to mature lamin A, in INS-1 832/13 cells and human islets [12, 13]. We also demonstrated that lamin undergoes carboxymethylation in pancreatic β -cells [15]. Based on data accrued from multiple investigations, we concluded that inhibition of C-terminal cysteine modifications of lamin-B might result in disruption of nuclear assembly, leading to further propagation of apoptotic signals, including DNA fragmentation and chromatin condensation [7, 13, 14]. However, this model has not been validated in pancreatic β -cells, specifically in the context of aberrant protein prenylation that we have described in the islet in pathological states such as glucolipotoxicity and ER stress (below).

We recently reported significant defects in protein prenylation pathway in pancreatic β -cells (human islets, rodent islets and INS-1 832/13 cells) exposed to metabolic stress conditions, including glucolipotoxicity and ER stress [16]. Specifically, we demonstrated a marked reduction in FTase and GGTase activities and subsequent accumulation of unprenylated G-proteins (Rap1) in pancreatic β -cells under these conditions. More importantly, inhibition of prenylation also led to paradoxical activation of Rac1 in all three types of insulin-secreting cells studied. Along these lines, it has been shown that glucotoxic and ER-stress conditions promote activation of caspase 3 and degradation of nuclear lamins [16]. However, potential contributory roles of protein prenylation in the retention of mitochondrial and nuclear functions remain less understood. Therefore, we undertook the current investigation to assess roles of protein prenylation in caspase 3 activation and

Fig. 1. The cholesterol biosynthesis pathway. The formation of cholesterol begins with acetyl CoA and aceto-acetyl CoA generating 3-hydroxy 3 methyl-glutaryl CoA (HMG-CoA), followed by a series of steps catalyzed by several enzymes along with generation of a number of intermediate metabolites. HMG-CoA reductase, the rate limiting enzyme of this pathway, catalyzes the synthesis of mevalonate. Simvastatin, a hypolipidemic drug, has an inhibitory effect on HMG-CoA reductase, thereby blocking the subsequent steps which lead to cholesterol biosynthesis. It also prevents the formation of metabolites of this pathway, namely: farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) that are involved in post translational prenylation of several proteins (Rho, Rab, Ras). FTI-277, inhibits the farnesyl transferase (FTase), which catalyzes the addition of 15 carbon farnesyl moiety to target proteins (Ras, nuclear lamins), thereby affecting the processing of these proteins.

lamin B degradation in INS-1 832/13 cells exposed to Simvastatin, a known inhibitor of MVA biosynthesis as well as its downstream intermediates including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphates (GGPP). Biosynthesis of FPP and GGPP is catalyzed by FPP synthase (FPSS) and GGPP synthase (GGPS), respectively. As depicted in Fig. 1, FPP and GGPP serve as substrates for farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase), respectively. The FTase mediates incorporation of FPP into nuclear lamins, small GTPases, such as Ras, and certain γ -subunits of heterotrimeric G-proteins. In contrast, GGPP serves as the substrate for GGTase-I and GGTase-II, which mediate geranylgeranylation of Rho and Rab G-proteins, respectively (Fig. 1). In addition to Simvastatin, we have employed FTI-277, a known inhibitor of FTase [17], to further assess contributory roles of farnesylation in lamin processing, and metabolic cell viability (Fig. 1). Data from our current investigations further support our original proposal that optimal prenylation is requisite for islet β -cell survival and function [17].

Materials and Methods

Materials

Antisera directed against cleaved caspase 3, total and phospho-ERK1/2 [Thr202/Tyr204] were obtained from Cell Signaling (Danvers, MA). Antibodies for lamin B, total and phospho-p38MAPK were

from Santa Cruz Biotechnology (Santa Cruz, CA). FTI-277 was from Tocris Biosciences (Minneapolis, MN). Simvastatin, antibody for β -actin, metabolic cell viability assay (MTT) kit and all other reagents employed in the current studies were from Sigma Aldrich (St. Louis, MO).

Insulin-secreting INS-1 832/13 cells and incubation conditions

INS-1 832/13 cells (kindly provided by Dr. Chris Newgard). Original investigations from Dr. Newgard's laboratory have isolated INS-1-derived β -cell lines that exhibited robust ATP-sensitive K^+ channel-dependent and -independent glucose-stimulated insulin secretion. Of these cell lines, the INS-1 832/13 cell line secreted greater than 10-fold insulin in response to physiological concentrations of glucose [18]. In addition, glucose-induced insulin secretion in these cells was potentiated by isobutylmethylxanthine, oleate/palmitate, glucagon-like peptide 1 and tolbutamide. A membrane depolarizing concentration of KCl (30 mM) also promoted insulin secretion from INS-1 832/13 cells. Since its development, numerous laboratories, including our own have employed this cell line in studies related to islet function including insulin secretion, proliferation and apoptosis. In the current studies, INS-1 832/13 cells were cultured in RPMI-1640 medium containing 10% heat inactivated FBS supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin, 1 mM sodium pyruvate, 50 μ M 2-mercaptoethanol and 10 mM HEPES (pH 7.4). The cultured cells were subcloned twice weekly following trypsinization and passages 53–61 were used for the study.

To assess the roles of MVA biosynthetic pathway, in general, and protein farnesylation pathway, in particular, we employed Simvastatin and FTI-277, respectively (Fig. 1). In these studies, INS-1 832/13 cells were incubated in the absence (diluent) or presence of simvastatin (0–30 μ M) and FTI-277 (15 μ M) for different time intervals as described in the text. Earlier studies have demonstrated that both of these agents, in the stated concentration range, significantly inhibited MVA and protein prenylation pathways in a variety of cells, including the islet β -cell [16, 17, 19–22].

Isolation of pancreatic islets

Islets from normal 6 week-old male Sprague–Dawley rats (Harlan Laboratories, Oxford, MI) were isolated by the collagenase digestion method [12, 16, 17]. All protocols, including isolation of pancreatic islets from rats, were reviewed and approved by the Wayne State University and John D. Dingell VA Medical Center Institutional Animal Care and Use Committees.

Western blotting

INS-1 832/13 cells (cultured overnight in RPMI media containing 2.5 mM glucose and 2.5% fetal bovine serum along with FTI-277 in certain experiments) and rat islets were incubated in the presence of low glucose (2.5 mM), FTI-277 (10 μ M) or Simvastatin (15 or 30 μ M) for 24 hrs. as indicated in the text. At the end of the incubation period the cells were harvested and lysed in RIPA buffer containing 1 mg/ml protease inhibitor cocktail, 1 mM NaF, 1 mM PMSF and 1 mM Na_3VO_4 . Cellular lysate proteins (30–50 μ g) were separated by SDS-PAGE and electro-transferred to a nitrocellulose membrane. The membranes were then blocked with 0.1 % Casein in 0.2X PBS for 1 hr. at room temperature. Blots were then incubated overnight at 4 °C with appropriate primary antibody in 0.2X PBS-T containing 0.1 % casein. The membranes were washed 5X for 5 min each with PBS-T and probed with appropriate IRDye® 800CW secondary antibody in 0.1 % casein in PBS-T at room temperature for 1 hr. After washing, the immune complexes comprised of the target proteins, were detected under Odyssey® Imaging Systems.

Subcellular phase partitioning using Triton X-114

INS-1 832/13 cells were cultured overnight in RPMI media containing 2.5 mM glucose and 2.5% fetal bovine serum and FTI-277 (10 μ M), cells were then treated with media containing low glucose (2.5 mM; LG) in the absence and presence of FTI-277 (10 μ M) for 24 hrs. Cell lysates were homogenized in a homogenization buffer (20 mM Tris-HCl, pH 7.5, 0.5 mM EGTA, 2 mM $MgCl_2$ and protease inhibitor cocktail) and subjected to a single-step centrifugation at 100,000g for 60 min at 4°C in order to separate the cytosol (supernatant) and the membrane (pellet) fractions. The membrane fraction was then subjected to hydrophobic and hydrophilic phase partitioning using Triton X-114 according to the method we described in [23, 24].

Metabolic cell viability assay

INS-1 832/13 cells were cultured in 96-well plates and treated with low glucose (2.5 mM; LG) or FTI-277 (10 μ M) or Simvastatin (15 or 30 μ M). After 24 hrs., cell viability was assessed by using a colorimetric assay with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) which measures the reduction of MTT into the purple formazan crystals by metabolically active cells at 570 nm [12].

Cell morphology

INS-1 832/13 cells, cultured overnight in RPMI containing 2.5 mM glucose and 2.5% fetal bovine serum, were further treated with media containing low glucose (2.5 mM; LG) alone and in the presence of diluent (DMSO), FTI-277 (10 μ M) or Simvastatin (15 and 30 μ M) for 24 hrs. Changes in cell morphology were determined under an Olympus IX71 microscope.

Statistical analysis

The statistical significance of the differences between the experimental conditions was determined by t-test or ANOVA where appropriate. *P* values < 0.05 were considered significant.

Results

As stated above, we have recently reported structural abnormalities in lamins in human islets, rodent islets and INS-1 832/13 cells following long-term exposure to glucotoxic conditions. Our findings indicated increased degradation of lamin B in pancreatic β -cells under these conditions [7, 12-14]. Furthermore, lamin degradation is mediated *via* caspase 3 activation since specific inhibitors of caspase 3 significantly attenuated lamin B degradation under these conditions [14]. As a logical extension, we investigated, herein, potential regulatory roles for protein prenylation in pancreatic β -cell function, including integrity of nuclear lamins. Data depicted in Fig. 2 (Panel A) demonstrate that incubation of INS-1 832/13 cells with Simvastatin (15 μ M; 24 hrs.) significantly induced caspase 3 activation and lamin B degradation. Data from multiple experiments affirm the hypothesis that inhibition of MVA and its downstream isoprenoids [FPP and GGPP] leads to caspase 3 activation (Fig. 2; Panel B) and nuclear lamin B degradation (Fig. 2; Panel D). Furthermore, incubation of INS-1 832/13 cells with Simvastatin (15 or 30 μ M), but not the diluent (DMSO) induced clear morphological changes (cell rounding) in these cells (Fig. 2; Panel C); these data further validate the original hypothesis that protein prenylation plays significant regulatory roles in cell morphology and cytoskeletal arrangements and architecture [24, 25]. Data depicted in Fig. 2 (Panel E) further confirm these findings of increased caspase 3 activation and nuclear lamin B degradation in primary rat islets exposed to Simvastatin.

Previous observations from our laboratory in INS-1 832/13 cells, rodent and human islets have demonstrated regulatory roles for Rac1, a geranylgeranylated protein, in the activation of stress kinases, such as p38MAPK under glucotoxic conditions [22]. Pharmacological inhibition of Rac1, observed under glucotoxic conditions, markedly attenuated p38MAPK activation [22]. Furthermore, we have demonstrated requirement protein farnesylation in glucose-induced activation of ERK1/2, a signaling kinase involved in β -cell proliferation [17]. In the current study, we assessed the effects of MVA (and FPP/GGPP) depletion on p38MAPK and ERK 1/2 in INS-1 832/13 cells exposed to Simvastatin. Data in Fig. 3 (Panels A and B) demonstrate significant activation of p38MAPK in cells exposed to simvastatin, suggesting that forced inactivation of prenylation pathway results in activation p38MAPK. In addition, compatible with our earlier observations demonstrating requirement for protein farnesylation in ERK1/2 activation, we noticed, herein, that exposure of INS-1 832/13 cells to Simvastatin led to inhibition of ERK1/2 activation (Fig. 3; Panels C and D). Taken together, these findings suggest regulatory roles for MVA and its isoprenoid derivatives, in mediating signaling steps involved in β -cell function and survival.

To conclusively demonstrate that inhibition of farnesylation of proteins (e.g., lamin B) would result in mitochondrial and nuclear dysregulation (as above), we studied the effects of

Fig. 2. Exposure of INS-1 832/13 cells and rat islets to Simvastatin leads to increased activation of caspase 3 and subsequent degradation of lamin B along with changes in cell morphology. Panel A: INS-1 832/13 cells were cultured in RPMI media containing 2.5 mM glucose (LG) or with 15 μM Simvastatin (Sim) for 24 hrs. Lysate proteins were resolved by SDS-PAGE and transferred to a nitrocellulose membrane and then probed for cleaved caspase 3 and lamin B. Uniform protein loading was ensured by re-probing the blots with antibody against β-actin. The statistical significance of the differences between the control and experimental conditions was determined by t-test. Panel B and C represent mean ± SEM values from three independent experiments, and are expressed as fold change in caspase 3 activation (Panel B) and lamin B degradation (Panel C). *P<0.05 vs. LG. Panel D: INS-1 832/13 cells, cultured overnight in RPMI containing 2.5 mM glucose and 2.5% fetal bovine serum, were further treated with media containing low glucose (2.5 mM; LG) alone and in the presence of diluent (DMSO), Simvastatin (15 μM and 30 μM; Sim) for 24 hrs. Changes in cell morphology were visualized by light microscopy. Panel E: Rat islets were incubated in a medium containing low glucose (2.5 mM; LG) in the absence and presence of simvastatin (15 or 30 μM; Sim) for 24hrs. Caspase 3 activation and lamin B degradation were determined by Western blotting. This Fig. is a representative blot from two independent experiments. Equal protein loading was ensured by re-probing the membrane with anti β-actin.

FTI-277, a known inhibitor of FTase (Fig. 1), on caspase 3 activation and lamin B degradation in INS-1 832/13 cells. For comparison, we also included a high glucose (HG; glucotoxic) condition in these experiments to determine the magnitude of effects of FTI-277 and HG in caspase 3 activation and lamin B degradation. Data in Fig. 4 (Panel A) suggest a marked

Fig. 3. Exposure of INS-1 832/13 cells to Simvastatin increases phosphorylation of p38 MAP Kinase while markedly suppressing the phosphorylation of ERK1/2. Lysates from INS-1 832/13 cells incubated in RPMI media containing 2.5 mM glucose (LG) alone and with 15 μ M Simvastatin (Sim) for 24 hrs. were resolved by SDS-PAGE and transferred to nitrocellulose membranes by wet transfer. The blots were probed for phosphorylated p38MAPK and total p38MAPK (Panel A), phospho-p44/42 ERK1/2 and total p44/42 ERK1/2 (Panel C). Band densities were calculated by densitometry. The statistical significance of the differences between the control and experimental conditions was determined by t-test. Data provided in Panels B and D represent mean $\pm$ SEM from three independent experiments, and are expressed as fold change in p38 MAPK phosphorylation (Panel B) and p44/42 ERK1/2 phosphorylation (Panel D). *P<0.05 vs. LG.

increase in caspase 3 activation and lamin B degradation in INS-1 832/13 cells incubated in the presence of either FTI-277 or HG. Pooled data from multiple experiments are provided in Fig. 4 (Panels B and C). These findings suggest nearly similar degrees of caspase 3 activation and lamin B degradation under both conditions. We next assessed alterations in the distribution of active caspase 3 and degraded lamin B in INS-1 832/13 cells exposed to either basal glucose or FTI-277. Total hydrophobic and hydrophilic compartments from these cells were isolated using the Triton X-114 phase separation method as we described in [23, 24]. Data in Fig. 4 (Panel D) indicated relatively low levels of active caspase 3 and degraded lamin B under basal conditions. More importantly, they both seem to be associated with the hydrophobic compartment. Interestingly, however, in FTI-277-treated cells, we noticed significantly higher abundance of active caspase 3; also associated with the hydrophobic compartment. In addition, a marked increase in the relative abundance of lamin-B was noticed in FTI-277-treated cells. Further, a much higher abundance of degraded lamin B was seen in the soluble compartment. Taken together, these findings suggest “leakage” of degraded lamin B to the soluble compartment due to the collapse of nuclear integrity under these conditions (see Discussion). Interestingly, unlike Simvastatin-treated cells (Fig. 2; Panel D), no detectable changes in the morphology of cells treated with FTI-277 were noted (Fig. 4; Panel E). These findings raise an interesting possibility that inhibition of prenylation of Rho G-proteins (e.g., Rac1) *via* inhibition of GGPP generation in Simvastatin-treated cells could contribute to cell rounding as reported by us and others previously [26, 27]. Lastly, metabolic cell viability measurements in FTI-277-treated cells indicated nearly 12% loss in cell viability (Fig. 4; Panel F). It is noteworthy that the degree of loss in cell viability is

Fig. 4. FTI-277 induces caspase-3 activation, lamin degradation and loss in cell viability in INS-1 832 cells. Panel A: INS-1 832/13 cells were incubated in a medium containing low glucose (2.5 mM; LG) in the absence and presence of FTI-277 (10 μM) for 24 hrs. Caspase 3 activation and lamin B degradation for each condition was determined by Western blotting (Panel A). Equal protein loading was ensured by re-probing the membrane with anti β-actin. Intensity of protein bands was quantified by densitometry. Data represent mean ± SEM from three independent experiments, and are expressed as fold change in caspase 3 activation (Panel B) and lamin B degradation (Panel C). *P<0.05 vs. LG. Panel D: Lysates from INS-1 832/13 cells treated with low glucose (2.5 mM) in the absence and presence of FTI-277 (10 μM) were subjected to a single-step centrifugation to separate the supernatant cytosolic (Cyto) and the membrane-pellet (Mem) fractions followed by hydrophobic (HPB) and hydrophilic (HPL) phase partitioning with Triton X-114 detergent [23,24]. The lysate proteins were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was probed for cleaved caspase 3 and lamin B. A representative blot from two independent experiments is shown here. Panel E: INS-1 832/13 cells were treated with media containing low glucose (2.5 mM; LG) alone and in the presence of diluent (DMSO), FTI-277 (10 μM) for 24 hrs. Changes in cell morphology were visualized by light microscopy. Panel F: INS-1 832/13 cells were treated with low glucose (2.5 mM; LG) in the absence and presence of FTI-277 (10 μM). After 24 hrs. cell viability was assessed by the MTT assay. Data represent mean ± SEM and are expressed as percent cell viability. *P<0.05 vs. LG.

comparable to what we observed in INS-1 832/13 cells exposed to glucotoxic conditions (i.e., 13 and 19 % reduction in metabolic cell viability in these cells following exposure to glucotoxic conditions at 24 and 48 h, respectively; ref [12].). Taken together, our findings from the studies described above suggest that depletion of endogenous MVA pools (with Simvastatin) or inhibition of FTase (with FTI-277) results in caspase 3 activation and lamin B degradation and loss in cell viability in INS-1 832/13 cells.

Discussion

The main objective of this study was to understand the impact of inhibition of protein prenylation on caspase 3 activation and lamin B degradation in pancreatic β -cells. We have employed two pharmacological inhibitors to address this question. Simvastatin was used to deplete intermediates of cholesterol biosynthetic pathway including MVA and its downstream isoprenoids, such as FPP and GGPP (Fig. 1). In addition, we used FTI-277, a known inhibitor of FTase, which is involved in farnesylation of lamins. Salient findings from these studies suggested that exposure of pancreatic β -cells to Simvastatin promotes (i) caspase 3 activation; (ii) lamin-B degradation; (iii) cell rounding; (iv) activation of p38MAPK; and (v) inhibition of ERK1/2 phosphorylation. Our findings also indicated that inhibition of FTase activity (using FTI-277) results in: (i) activation of caspase 3; (ii) degradation of lamin B; (iii) significant accumulation of degraded lamin in the cytosolic fraction; and (iv) loss in metabolic viability in INS-1 832/13 cells. Together, we provide evidence to implicate roles for protein prenylation in mitochondrial and nuclear dysregulation in pancreatic β -cells.

What are the implications of these findings to metabolic alterations seen in these cells exposed to metabolic stress condition including glucotoxicity, lipotoxicity and ER stress? It is likely that aberrant prenylation of proteins could contribute to these metabolic defects. For example, Veluthakal and associates [16] have recently demonstrated functional inactivation of FTase/GGTase activities under conditions of metabolic stress. This, in turn, led to significant accumulation of unprenylated, but paradoxically active G-proteins (Rac1) under these conditions. It is likely that functional defects in the prenylation pathway seen under these conditions (similar to those reported in these studies) could favor metabolic changes in the nuclear compartment including increasing the susceptibility of lamins for degradation by pro-apoptotic caspases. Indeed, our recent observations support such a formulation. They are discussed briefly below.

First, we have recently demonstrated that incubation of INS-1 832/13 cells, normal rat islets or human islets under glucotoxic conditions (20 mM; 12-48 h) results in the degradation of native lamin B leading to accumulation of the degraded products in non-relevant cellular compartments, including cytosol [14]. Furthermore, the effects of high glucose on caspase 3 activation and lamin B degradation were mimicked by thapsigargin, a known inducer of ER stress. Nifedipine, a known blocker of calcium channel activation, inhibited high glucose-induced caspase 3 activation and lamin B degradation in these cells. Lastly, 4-Phenyl butyric acid, a known inhibitor of ER stress, markedly attenuated glucose-induced CHOP expression (ER stress marker), caspase 3 activation and lamin B degradation. Based on these findings we concluded that glucotoxic conditions promote caspase 3 activation and lamin B degradation, which may, in part, be due to increased ER stress under these conditions. These studies also provided evidence to support beneficial effects of calcium channel blockers against metabolic dysfunction of the islet β -cell induced by hyperglycemic conditions.

It should be kept in mind, however, that other signaling events could also contribute to the defective metabolic fate of the islet β -cell under metabolic stress conditions. For example, published evidence also suggests significant alterations in the (de)phosphorylation of lamin under the duress of metabolic insults including lipotoxicity and exposure to pro-inflammatory cytokines. In this context, studies by Eitel et al. demonstrated critical roles for PKC δ in free fatty acid (FFA; palmitate)-mediated apoptosis in insulin-secreting RIN1046-38 cells [28]. They reported rapid (within 15 min) translocation of protein kinase δ (PKC δ) to

the nuclear fraction following exposure to FFA culminating in lamin B1 disassembly. They also reported significant attenuation of FFA-induced apoptosis in these cells overexpressing a dominant negative mutant of PKC δ . Based on these findings, the authors concluded that nuclear translocation and kinase activity of PKC δ are both necessary for FFA-induced apoptosis. Studies by Veluthakal and coworkers [29] have investigated functional status of lamin B in INS-1 cells exposed to pro-inflammatory cytokines. They identified an okadaic acid-sensitive protein phosphatase 2A-like phosphatase in the nuclear fraction in INS-1 cells. Immunoblotting and vapor-phase equilibration assays revealed expression and carboxymethylation of the catalytic subunit of protein phosphatase 4 (PP4c) in the nuclear fraction. Exposure of INS-1 cells to IL-1 β resulted in a marked increase in nitric oxide release with concomitant reduction in the degree of expression, the carboxymethylation and the catalytic activity of only PP4 in the nuclear fraction. Furthermore, immunoprecipitation studies suggested potential complexation of the catalytic subunit of PP4 with nuclear lamin-B, a key regulatory protein involved in the nuclear envelope assembly. Based on these findings, these investigators proposed that IL-1 β -mediated inhibition of PP4 activity might result in the retention of lamin-B in its phosphorylated state, which is essential for its degradation by caspases leading to the apoptotic demise of the β -cell. Therefore, in addition to defective prenylation, it is likely that other regulatory mechanisms, such as phosphorylation-dephosphorylation might increase the susceptibility of lamins for degradation of caspases culminating in cellular dysfunction and apoptosis.

Lastly from a clinical standpoint, as stated above, aberrant farnesylation of lamins, especially lamin A, has been linked to the onset of a number of diseases affecting the striated muscle [e.g. Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy], adipose tissue [e.g. Dunnigan-type familial partial lipodystrophy] and could result in abnormal senescence and growth deformities (e.g. Progeria syndrome; refs [7, 9-11]). Clearly, additional studies (basic as well as clinical) are needed to further assess potential impact of defective prenylation of lamins in glucolipotoxicity in the lamin-related pathologies in diabetes [7, 13]. It is noteworthy that, recent studies by Yang et al [30]. have reported novel roles for RhoA prenylation in cardiac and vascular remodeling in spontaneously hypertensive rats. Specifically, they demonstrated that alendronate, a known inhibitor of FPPS, treatment significantly suppressed cardiac hypertrophy and fibrosis in spontaneously hypertensive rats. Furthermore, alendronate treatment markedly suppressed FPP and GGPP levels, RhoA prenylation and activation in heart and aorta. These observations further substantiate key regulatory roles of protein prenylation in cellular function and dysfunction.

Conclusion

We conclude that inactivation of prenylation pathway, either by depletion of MVA and its downstream isoprenoids (FPP and GGPP) in pancreatic β -cells leads to metabolic defects leading to activation of stress kinases (p38MAPK), inactivation of signals involved in β -cell proliferation (ERK1/2) culminating in caspase 3 activation. We also conclude that inhibition of farnesylation increases susceptibility of non-farnesylated proteins, such as lamin B, for degradation by pro-apoptotic caspases, such as caspase 3 leading to loss in metabolic cell viability. Together, these findings affirm our working model that functional inactivation of prenylation pathway in islet β -cells under the duress of metabolic stress and diabetes leads to increased stress kinase activation [21, 22, 31] dysregulation of metabolic events that are essential for islet β -cell function, including proliferation and survival [13].

Abbreviations

ER stress (Endoplasmic reticulum stress); ERK1/2: (extracellular signal-regulated kinases 1/2); FTase (farnesyltransferase); FTI-277 (farnesyltransferase inhibitor-277); FPP

(farnesylpyrophosphate); FPPS (farnesylpyrophosphate synthase); GGTase (geranylgeranyl-transferase); GGPP (geranylgeranylpyrophosphate); GGPPS (geranylgeranylpyrophosphate synthase); MVA (mevalonic acid); PP4 (protein phosphatase-4); PP4c (catalytic subunit of PP4); PKC δ (protein kinase δ); p38MAPK (p38 mitogen-activated protein kinases).

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Disclosure Statement

The authors declare that there is no conflict of interest regarding publication of this paper.

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