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Guest Editors: Romano Regazzi, Stephane Dalle, and Amar Abderrahmani

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Editorial Board

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Editorial

Compensatory Mechanisms of Pancreatic Beta Cells: Insights into the Therapeutic Perspectives for Diabetes

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Diabetes is one of the leading causes of premature mortality worldwide and has become one of the major health challenges of the 21st century. Diabetes develops when insulin production is insufficient to compensate for the increased insulin requirements elicited by insulin resistance. Diabetes prevalence has risen dramatically during the last 20 years in parallel to the pandemic of obesity. Although obesity is the first diabetes risk factor, many obese and insulin resistant people do not suffer from diabetes. This situation is thought to result from the capacity of beta cell to adapt its mass and function in order to produce enough insulin to cover the needs of the organism. Beta cell adaptation is a physiological process that occurs efficiently from birth to early childhood periods and during pregnancy. Beta cell functional mass adaptation relies on both increased intrinsic insulin secretory capacity of the cell and beta cell mass expansion and preservation of cell survival against apoptosis. Deciphering the key mechanisms that account for such beta cell plasticity would help understanding the pathophysiology of diabetes, paving the way for the generation of innovative antidiabetic compounds aiming at preventing the development and the progression of the disease. In this respect, this special issue contains two regular articles and four reviews that highlight some of the mechanisms involved in the control of beta cell mass and function.

Beta cell mass adaptation relies on neogenesis and, possibly, on an increase in beta cell proliferation. Although so far only few studies have confirmed a consistent replication of human adult beta cells, this phenomenon has been demonstrated to play a major role in rodent beta cell mass expansion. Beta cell proliferation involves a tight control and interconnectedness in the expression of activators and inhibitors of the cell cycle. In this special issue, the paper entitled "*Role of Ink4a/Arf locus in beta cell mass expansion under physiological and pathological conditions*" by E. Salas et al. reports the pieces of evidence pointing to a role for the two tumor suppressors $p16^{ink4a}$ and $p14^{Arf}$ encoded by the Ink4a/Arf in beta cell expansion. In this paper, the authors provide an insightful description of their mode of action and their regulation under diabetogenic and physiological conditions. Induction of the unfolded protein response, also referred to as endoplasmic reticulum stress (ER), is a key cellular phenomenon that governs beta cell compensation. Induction of ER stress is required for the effects of glucagon like-peptide 1 (GLP-1) on the potentiation of glucose-induced insulin secretion and beta cell survival. The review entitled "*Role of the unfolded protein response in β cell compensation and failure during diabetes*" by N. Rabhi et al. sheds light on the key signaling pathways of the ER stress involved in the regulation of beta cell function and survival under physiological and diabetes condition.

Mechanisms involved in the control of glucose-induced insulin secretion are attractive targets for antidiabetic therapy. Closure of ATP-dependent potassium channel is a key

event that is required for glucose-induced insulin secretion. This channel is closed by glinides, a class of oral antidiabetic agents. As a consequence of the drug action, the sensitivity of beta cells to elevated glucose levels is increased and, thereby, the early-phase prandial insulin response is enhanced. The goal of the paper entitled "*Comparison of metformin and repaglinide monotherapy in the treatment of new onset type 2 diabetes mellitus in China*" by J. Ma et al. is to compare the efficiency of metformin and repaglinide monotherapies in the glycemic control of newly diagnosed diabetic Chinese patients. The study reports a decrease of glycated hemoglobin and, finally, a better glycemic control in the diabetic patients treated with repaglinide when compared to metformin. This beneficial effect is associated with increased beta cell function. This paper underlines the effectiveness of approaches targeting the beta cell as the first medication to rapidly improve the glycemic control. While the classes of stabilized GLP-1 mimetics and dipeptidyl peptidase-IV inhibitors aim to potentiate glucose-induced insulin secretion by triggering the GLP-1 receptor signaling, they are not first-line drugs for lowering blood glucose. However, there are numerous clues underlining their potent effects in preventing a steady decline of beta cell functions overtime and, consequently, the progression of diabetes. Increased GLP-1 levels may contribute to functional beta cell mass adaptation in obesity and pregnancy and, importantly, may also protect beta cells against the harmful effects of diabetogenic factors including proinflammatory cytokines. The beneficial effects of incretins such as GLP-1 on beta cells are thought to represent also one of the important mechanisms whereby bariatric surgery in obese diabetic patients permits to improve glycemia aside from massive weight loss. The H. Ezanno et al. paper entitled "*JNK3 is required for the cytoprotective effect of exendin 4*" highlights the key role of the beta cell restrictive c-Jun amino terminal kinase 3 (JNK3) in relaying the protective effect of the exendin 4 GLP-1 mimetic against cell death elicited by proinflammatory cytokines. The study provides some lines of evidence that JNK3 could be an attractive drug target.

Induction of the inducible cAMP early repressor (ICER) is part of the adaptive mechanisms through which GLP-1 and its mimetics allow insulin secretion to return to basal state. ICER is a passive repressor that inhibits the expression of genes containing a cAMP response element (CRE) in their promoter. Upon silencing its target genes, the level of ICER needs to be reduced for preserving beta cell integrity. Several studies reviewed in the paper entitled "*Decompensation of β-cells: when pancreatic β-cells are on ICE(R)*" by R. Salvi and A. Abderrahmani have shown that abnormal expression of ICER underlies dysfunction and death of insulin-secreting cells under diabetogenic conditions, thus emphasizing the requirement for a tightly controlled expression of ICER for beta cell compensation.

The literature demonstrating a role for microRNAs in beta cell compensation during obesity and pregnancy is emerging. In this special issue, the paper entitled "*Role of microRNAs in islet beta-cell compensation and failure during diabetes*" by V. Plaisance et al. provides an overview of the potential involvement of these small noncoding RNAs in beta cell mass expansion and failure occurring during normal development and in response to metabolic changes and diabetogenic conditions including the exposure to cytokines, oxidized LDL, and palmitate.

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Research Article

JNK3 Is Required for the Cytoprotective Effect of Exendin 4

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Preservation of beta cell against apoptosis is one of the therapeutic benefits of the glucagon-like peptide-1 (GLP1) antidiabetic mimetics for preserving the functional beta cell mass exposed to diabetogenic condition including proinflammatory cytokines. The mitogen activated protein kinase 10 also called c-jun amino-terminal kinase 3 (JNK3) plays a protective role in insulin-secreting cells against death caused by cytokines. In this study, we investigated whether the JNK3 expression is associated with the protective effect elicited by the GLP1 mimetic exendin 4. We found an increase in the abundance of JNK3 in isolated human islets and INS-1E cells cultured with exendin 4. Induction of JNK3 by exendin 4 was associated with an increased survival of INS-1E cells. Silencing of JNK3 prevented the cytoprotective effect of exendin 4 against apoptosis elicited by culture condition and cytokines. These results emphasize the requirement of JNK3 in the antiapoptotic effects of exendin 4.

1. Introduction

Preservation of mechanisms underlying adaptation of beta cells mass and function is critical for glucose homeostasis, as the decline in functional beta cells mass is a key feature of the development of diabetes [1–5]. The incretin hormone glucagon-like peptide-1 (GLP1) plays an instrumental role in the control of beta cell mass and function [6–8]. Alteration of beta cell sensitivity to GLP1 is thought to contribute to the loss of functional beta cell mass in diabetes in both lean and obese individuals [9–11]. Beta cell abnormalities in the GLP1 sensitivity have been associated with a reduction in the GLP1 receptor expression in some animal models of diabetes [12, 13]. Administration of GLP1 improves beta cell survival in animal model of diabetes [14, 15]. A wealth of *in vitro* and *in vivo* studies show that this prosurvival effect is achieved by inhibiting beta cells apoptosis elicited by diabetogenic stressors such as proinflammatory cytokines [6, 16–23]. The effect achieved by the GLP1 and its analogs results from the activation of kinases and/or scaffold proteins, which in turn promote an antiapoptotic signaling cascade [6, 18, 19, 24–28]. One of the major kinases activated by GLP1 and its mimetic exendin 4 is the protein kinase B/AKT [16, 17, 27]. Activation of AKT by GLP1 results from the increased abundance of the insulin receptor substrate 2 (IRS2) [27]. In beta cells, the expression of IRS2 is controlled by the mitogen activated protein kinase 10 also called c-jun amino-terminal kinase 3 (JNK3) [29, 30]. Silencing of JNK3 by interference RNA dramatically reduces the IRS2 abundance in INS-1E cells [29]. As a result of JNK3 depletion an increase in cytokine-induced apoptosis ensues [29, 30]. In view of these data, the goal of this study was to investigate whether the JNK3 content is associated with beta cell protection achieved by the GLP1 mimetic exendin 4.

2. Material and Methods

2.1. Cell Culture, Human Islets Isolation, and Transfection. The rat insulin-secreting cell line INS-1E was maintained in RPMI 1640 medium supplemented with 10% fetal calf

serum (FCS) (PAA laboratories, GE Healthcare, Velizy-Villacoublay, France), 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, and 10 mM Hepes [31]. Human pancreases were harvested from adult brain-dead donors in accordance with French regulations and with the local Institutional Ethical Committee from the "Centre Hospitalier Régional et Universitaire de Lille." Pancreatic islets were isolated after ductal distension of the pancreas and digestion of the tissue as described previously [32]. All experiments were carried out at least on islets with a purity of and viability >80%. Purified islets were cultured in CMRL 1066 medium (Gibco BRL, Life Technologies) containing 0.625% free fatty acid human serum albumin (Roche Diagnostics), penicillin (100 μUI/mL), and streptomycin (100 μg/mL). The siRNA duplexes directed against JNK3 (siJNK3) or siRNA control against GFP (siGFP) were previously described [29–31]. The siRNA duplexes were introduced using the Lipofectamine 2000 (Life Technology, Saint Aubin, France) as described [29, 30].

2.2. Western Blotting Experiments. INS-1E and isolated human islets cells were scrapped in cold PBS buffer and cell pellets were incubated 30 min on ice in lysis buffer (20 mM Tris acetate pH 7, 0.27 mM sucrose, 1% Triton X-100, 1 mM EDTA, and 1 mM EGTA, 1 mM DTT) supplemented with antiproteases and antiphosphatases (Roche, Meylan, France). Cell lysates were centrifugated 15 min at 18,000 g and supernatants were used to analyze proteins. Protein extracts were solubilized in Laemmli buffer (40% glycerol, 20% β-mercaptoethanol, 8% SDS, 0.02% bromophenol blue, 0.25 mM Tris-HCl, pH 6.8) and denatured 10 min at 95°C before loading onto the gel. Proteins were separated on 10% SDS-polyacrylamide gel and electrically blotted to nitrocellulose membranes. The proteins were detected after an overnight incubation of the membrane at 4°C with the specific primary antibodies against JNK3 (dilution 1 : 1000; Cell Signaling Technology, MA, USA), JNK2 (dilution 1 : 1000; Cell Signaling Technology, MA, USA), β-actin (1 : 5000; Sigma, Saint Quentin, France), or α-tubulin (1 : 5000; Sigma, Saint Quentin, France), diluted in buffer containing 0.1% Tween 20 with either 2% milk (for JNK3) or 5% BSA (for JNK2) or 5% milk (for β-actin and α-tubulin). Proteins were visualized with IRDye800 or IRDye700 (Eurobio, Les Ulis, France) as secondary antibodies. Quantification was performed using the Odyssey infrared imaging system (Eurobio) [29, 30].

2.3. Apoptosis Assay. Apoptosis was evaluated in cells transfected with the siRNAs and exposed to a cytokine cocktail (R&D Systems, Minneapolis, MN, USA) of rat IL-1β (10 ng/mL), mouse TNFα (25 ng/mL), and rat IFNγ (150 ng/mL) for 24 h. Apoptosis was determined by scoring cells displaying pycnotic nuclei (visualized with Hoechst 33342) [31]. The counting was performed blindly by three different experimenters.

2.4. Statistical Analysis. ANOVA was used for statistical significance, followed by the post hoc Bonferroni test (Dunnett's test) when experiments included more than two groups. The level of significance was set at $P < 0.05$ (SAS statistical package; SAS, Carry, NC).

3. Results

3.1. Exendin 4 Increases the JNK3 Abundance in Isolated Human Islets and INS-1E. Several studies, including ours, have shown that the GLP1 receptor agonists prevent apoptosis elicited by prolonged exposure with cytokines [21, 23, 31]. Typically, the cytoprotective effect of the GLP1 mimetic is achieved through induction of key prosurvival proteins [6, 25]. In this regard we questioned whether exendin 4 increased the abundance of JNK3. We found that exposure of isolated human islets cells to exendin 4 elevated the JNK3 abundance as revealed by western blotting analysis (Figure 1(a)). The increase in the JNK3 protein started as early as 2 hr and declined after 4 hr (Figure 1(a)). Induction of JNK3 by exendin 4 was observable at 10 nM but was optimal at 50 nM (Figure 1(b)). Western blotting experiment confirmed elevation of JNK3 protein by exendin 4 in INS-1E cells (Figure 1(c)). However, induction of JNK3 by the GLP1 receptor agonist came later (after 4 hr of incubation) and persisted until 24 h treatment (Figure 1(c)).

3.2. JNK3 Is Required for the Cytoprotective Effects of Exendin 4 in INS-1E Cells. We next investigated whether JNK3 was required for the cytoprotective effect of exendin 4. To this end, INS-1E cells were transfected with the duplex siRNAs directed against JNK3 mRNA (siJNK3) [29, 30]. The latter efficiently and selectively silenced the expression of JNK3 in INS-1E cells (Figure 2(a)). As anticipated, incubation of the cells with cytokines for 24 hr elicited a 2-fold increase in apoptosis (Figure 2(b)). Exendin 4 efficiently reduced death evoked by culture conditions and cytokines (Figure 2(b)). As previously observed [29, 30], silencing of JNK3 potentiated cytokines-induced apoptosis (Figure 2(b)). In addition, diminution of JNK3 by siJNK3 abolished the protective effects accomplished by exendin 4 (Figure 2(b)). These data point out that JNK3 levels are pivotal for the coupling of exendin 4 and protection of cells against apoptosis evoked by cytokines.

4. Discussion

There is a growing body of evidence that the GLP1 and its mimetics trigger cytoprotective effects on beta cells by stimulating the abundance of antiapoptotic proteins [6, 17, 25–27, 31, 33]. Several reports have now delineated a role for JNK3 as a key player in protecting beta cells against apoptosis [29, 30]. A hallmark of this claim is that selective silencing of JNK3 increases apoptosis induced by cytokines [29, 30]. Inversely, we questioned whether the JNK3 content could be stimulated by the GLP1 mimetic exendin 4. We found that exposure of isolated human islets to exendin 4 increases the JNK3 protein content. Although the anti-apoptotic mechanisms activated by the GLP1 mimetics are globally similar between human islets and the rat insulin-secreting INS-1E cells [6, 17, 25, 26, 31], the spatial and

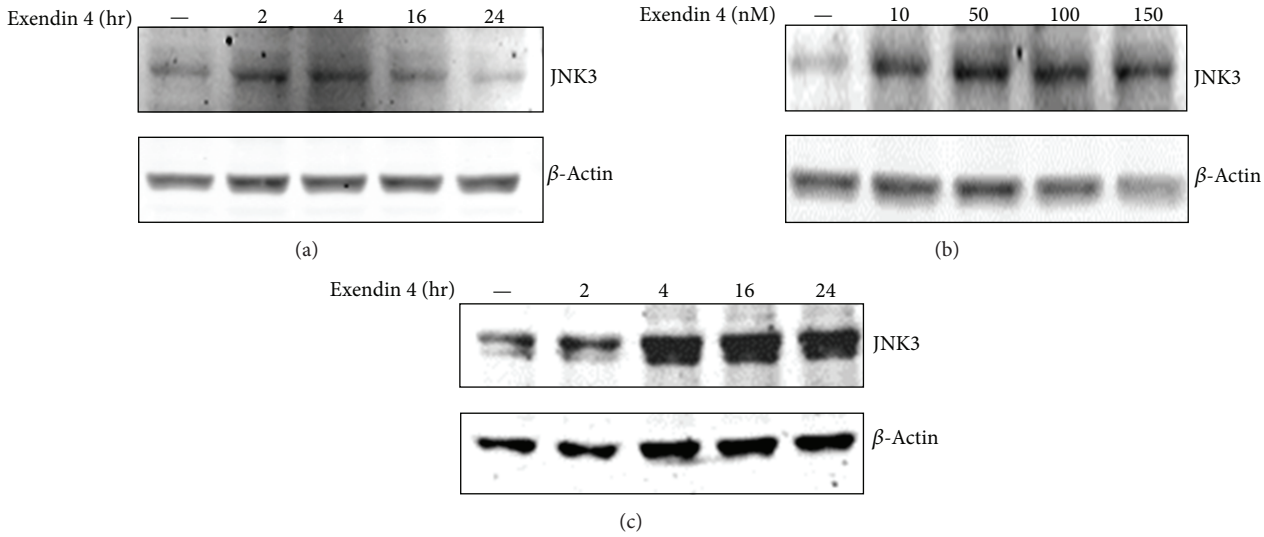

Figure 1: The effect of exendin 4 on the JNK3 content. JNK3 abundance in (a) isolated human islets (from three different donors) cultured with 50 nM exendin 4 for the indicated times or (b) with different exendin 4 concentrations for 4 hrs. (c) INS-1E cells cultured with 50 nM exendin 4 for the indicated times. For western blotting experiments, protein extracts (50 μg) were loaded into a polyacrylamide gel electrophoresis. Immunoblotting was achieved using the anti-JNK3 and anti-β-actin antibodies. The data is one representative experiment out of three.

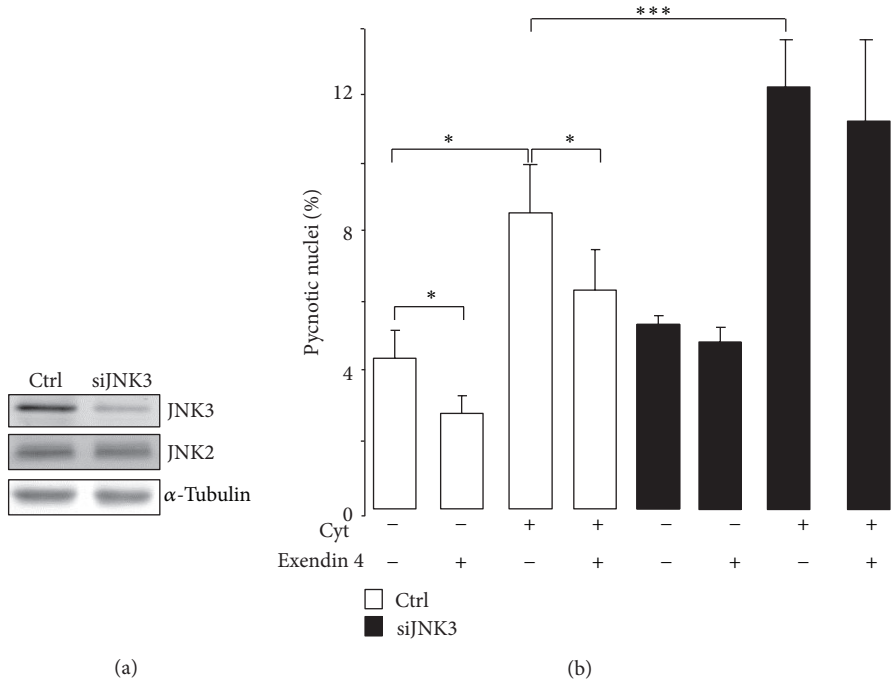

Figure 2: Impact of the JNK3 silencing on the protective effects of exendin 4. INS-1E cells were transfected with the siRNA duplex directed against JNK3 (siJNK3) or control siRNA (siGFP, Ctrl). (a) For western blotting analysis of the JNK3 level, total proteins were prepared 48 hr after transfection. Immunoblotting was done using the anti-JNK3, anti-JNK2, and anti-α-tubulin antibodies (b) for scoring death; the cells were preincubated 24 hr after transfection with 50 nM exendin 4 for 8 hr. The rate of apoptosis was scored by counting pycnotic nuclei in INS-1E cells exposed for 16 hr to the cocktail of cytokines including 10 ng/mL IL-1β, 15 ng/mL TNFα, and 150 ng/mL IFNγ. Results are expressed as mean ± SEM of 3 independent experiments. $^{*}P < 0.05$; $^{***}P < 0.001$.

temporal regulation of certain pathways evoked by GLP1 and its analogs may be species-specific. Different temporal activation of the antiapoptotic ERK pathway between isolated human islets and INS-1E cells has been shown in response to RF26a RFamide peptide [34]. While ERK is activated by the peptide in human islets and INS-1E cells, peptide-induced ERK activation is more prolonged in INS-1E cells [35]. One study has shown that beta cells behaviour in response to GLP1 is different between human and rodent islets [36]. GLP1 promotes cooperation and connectedness between beta cells within human islets whereas it does not do this in rodent cells [36]. This difference may elicit some changes in the spatial and temporal regulation of genes expression and pathways. In this regard, we observed that induction of JNK3 content by exendin 4 was faster and declined more rapidly in human islets when compared to INS-1E cells.

The induction of JNK3 in human islets and INS-1E cells led us to ask whether such phenomenon contributed to the protective effects elicited by exendin 4 against apoptosis. One clue was that the increase of JNK3 content in cultures of INS-1E cells for 24 h was associated with a significant reduction in apoptosis under normal culture condition. This antiapoptotic effect achieved by the GLP1 mimetic was abolished when JNK3 content was reduced by siRNA. We have previously published that cytokine treatment of INS-1E cells with cytokines worsens death caused by apoptosis [31]. The rise of death induced by cytokines is alleviated by coculturing the cells with exendin 4 [31]. The experiments unveiled that protection of INS-1E cells by exendin 4 against cytokine-induced apoptosis is abolished when the JNK3 abundance is attenuated.

Several key transcription factors and signalling proteins including protein kinase A (PKA), PKB/AKT, PKC-zeta, ERK, endoplasmic reticulum stress, and epidermal growth factor receptor are involved in the cytoprotective effects achieved by the GLP1 mimetics [6, 18, 19, 24–26]. Abdelli and coauthors have shown a reduction in the expression of insulin receptor substrate 2 (IRS2) and Akt activation upon silencing of JNK3 [29, 30]. However, JNK3 is mainly localized in the nucleus of beta cells [29, 30], suggesting that IRS2 cannot be the only target of the kinase. Future studies are needed to identify other targets of JNK3 that are required for the antiapoptotic effects of exendin 4. Such investigation could uncover novel protective pathways of beta cells and eventually lead to innovative antidiabetic therapeutic targets.

Conflict of Interests

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

Authors' Contribution

Hélène Ezanno and Valérie Pawlowski equally contributed to the work.

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Review Article

Role of the Unfolded Protein Response in β Cell Compensation and Failure during Diabetes

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Pancreatic β cell failure leads to diabetes development. During disease progression, β cells adapt their secretory capacity to compensate the elevated glycaemia and the peripheral insulin resistance. This compensatory mechanism involves a fine-tuned regulation to modulate the endoplasmic reticulum (ER) capacity and quality control to prevent unfolded proinsulin accumulation, a major protein synthetized within the β cell. These signalling pathways are collectively termed unfolded protein response (UPR). The UPR machinery is required to preserve ER homeostasis and β cell integrity. Moreover, UPR actors play a key role by regulating ER folding capacity, increasing the degradation of misfolded proteins, and limiting the mRNA translation rate. Recent genetic and biochemical studies on mouse models and human UPR sensor mutations demonstrate a clear requirement of the UPR machinery to prevent β cell failure and increase β cell mass and adaptation throughout the progression of diabetes. In this review we will highlight the specific role of UPR actors in β cell compensation and failure during diabetes.

1. Introduction

Type 2 diabetes (T2D) mellitus is a chronic metabolic disease with "epidemic" proportions. Its global prevalence was estimated to be 6.4% worldwide (285 million adults in 2010) and is predicted to rise to approximately 7.7% (439 million) by 2030 [1]. T2D is a multifactorial disorder resulting from an interaction between genetic and environmental conditions (sedentary lifestyle and Western diet) and characterized by a peripheral insulin resistance, hyperglycaemia, and pancreatic β cell dysfunctions. Two defects have been reported during diabetes development, a gradual deterioration of β cell functions and a reduction in pancreatic β cells mass. β cell failure is not limited to T2D but is rather a common feature of all forms of diabetes, including the autoimmune type 1 diabetes (T1D), autosomal dominant onset diabetes of young (MODY), Wolfram syndrome, and Wolcott-Rallison syndrome (WRS).

In the early stage of diabetes development, the response of pancreatic islets challenged by nutrients and/or insulin resistance is a hypersecretion of insulin to maintain normoglycaemia. To this end, an adaptative and compensatory response of β cells is required. The process of β cell compensation is a combination of β cell mass expansion and an increase of acute glucose-stimulated insulin secretion. Postmortem analyses of pancreas of nondiabetic obese patients show an increase of β cell volume, implying postnatal plasticity of β cell mass. Moreover the β cell compensation process is associated with an improved capacity of the secretory machinery to support increased insulin production. Subsequently, the production of large amounts of insulin by compensating islet β cells places a continuous demand on the ER for proper protein synthesis, folding, trafficking, and secretion. When the folding capacity of the ER is exceeded, misfolded or unfolded proteins accumulate in the ER lumen, resulting in ER stress.

The cytoprotective response to ER stress is the unfolded protein response (UPR). Paradoxically, UPR signalling activation leads to opposite cell fates, that is, adaptation/survival versus death. Increasing evidence links the endoplasmic reticulum (ER) stress to β cell deterioration and apoptosis [2, 3]. Recent experiments performed in db/db mice and ob/ob mice models at different times of disease progression revealed that the maintenance (or suppression) of adaptive UPR is associated with β cell compensation (or failure) in obese mice [4]. Moreover, Engin et al. recently showed a progressive loss of UPR mediator expression before the onset of diabetes in NOD mice [5]. The administration of the chemical chaperone tauroursodeoxycholic acid to rescue the deleterious ER stress response improved pathophysiological signs of diabetes with a recovery of β cell survival and adaptation to stress [5]. In addition, the authors showed a decline of the UPR mediator in both experimental models and T2D human islets, suggesting that decreased expression of β cell UPR actors can play a central role in β cell compensation and subsequently T2D occurrence [6].

2. The UPR Pathway

Three canonical ER resident molecules mediate UPR response, namely, protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6), which are maintained inactive by their association with the immunoglobulin heavy chain-binding protein (BiP, GRP78) in normal conditions (Figure 1(a)). The accumulation of unfolded proteins in the ER leads to the release of PERK, IRE, and ATF6 and their subsequent activation [7, 8]. The downstream signalling effectors from these pathways converge to the nucleus and activate UPR target genes, finally reducing the ER input (Figure 1(a)). Their action is bipartite, with an acute programme that attenuates the ER workload and a latent transcriptional one that builds ER capacity.

PERK is a type 1 ER transmembrane kinase with a stress sensing luminal N-terminal domain. During ER stress PERK phosphorylates the α-subunit of eIF2-α on serine 51 leading to a delivery inhibition of the initiator methionyl-tRNAi to the ribosome and ultimately resulting in global protein translation attenuation [9] (Figure 1(a)). This phosphorylation event directly contributes to the reduction of ER stress and protects cells from ER stress-mediated apoptosis [10]. Intriguingly, the mRNA transcription of UPR target genes is selectively activated by eIF2α phosphorylation, as these polycistronic mRNAs have inhibitory upstream open reading frames (uPRFs) and are thus preferentially translated by the ribosome. These include the bZiP transcription factor 4 (ATF4) that acts as a regulator of UPR target genes such as C/EBP-homologous protein (CHOP) and growth arrest and DNA damage inducible gene 34 (GADD34), as well as genes involved in the redox balance and amino acid synthesis [11]. GADD34 interacts with the catalytic subunit of protein phosphatase (PP1c) and controls the level of eIF2-α phosphorylation by a negative feedback loop [12], allowing the restoration of an UPR basal state once ER stress is resolved.

IRE1 is a central regulator of UPR. Like PERK, IRE1 is also a type 1 transmembrane kinase with an N-terminal luminal domain that senses ER stress signalling. Two homologues of IRE1 have been described, IRE1α and IRE1β. IRE1α is expressed ubiquitously, showing high expression levels in the pancreas and placenta [13], whereas IRE1β is only expressed in the intestinal epithelium and lung [14]. IRE1 possesses kinase as well as endoribonuclease activities. Once the ER stress is triggered, IRE1 activates its RNase domain through its dimerization and transautophosphorylation and causes an unconventional splicing by the removal of 26-nucleotide intron from the X-box binding protein 1 (XBP1) mRNA (Figure 1(a)). The subsequent spliced XBP1 (XBP1s) mRNA encodes a leucine zipper transcription factor with a high transcriptional activity that upregulates genes encoding ER protein chaperones, ER associated protein degradation (ERAD), and lipid biosynthetic enzymes [15, 16]. IRE1 has also a nonspecific RNase activity that degrades mRNAs localized near the ER membrane, thereby reducing protein import into the ER lumen [17]. High levels of ER stress also activate the kinase activity of IRE1 and initiate a signalling cascade of apoptosis signal-regulating kinase 1 (ASK1)/cJun amino terminal kinase (JNK), which can participate in the apoptotic cell fate [18].

ATF6 is an ER located type 2 transmembrane protein with a basic leucine zipper DNA binding domain (Figure 1(a)). Two ubiquitously expressed isoforms of ATF6 have been described, ATF6α and ATF6β [19]. Under ER stress conditions, ATF6α translocates from the ER to the Golgi apparatus, where it is cleaved by Site-1 protease and Site-2 protease (S1P/S2P). The newly generated cytosolic fragment migrates to the nucleus and activates UPR gene transcription [20, 21]. The exclusive or the combined action of cleaved ATF6α and XBP1s is able to activate all three ER stress response elements: ERSE, UPRE, and ERSE2 [22]. ATF6β was first described as a repressor of ATF6α [23]. However, mouse embryonic fibroblasts generated from ATF6β null mice did not show altered UPR gene induction, suggesting a minor role for ATF6β in ER stress response [24]. In contrast, ATF6α null mice have a significant alteration in their UPR gene expression profile, suggesting a central role for ATF6α in ER protein quality control and protection against ER stress [24]. As the double ATF6α/β knockdown is lethal, the authors suggested that ATF6α and ATF6β provide a complementary function during early development [24]. Moreover ATF6 activity is regulated by the Wolfram syndrome 1 (WFS1) protein, which targets ATF6 to the E3 ubiquitin ligase HRD1, consequently resulting in its ubiquitination and proteasomal degradation [25]. A number of other ER stress transducers that share a high sequence homology with ATF6 have been identified, such as Luman, OASIS, BBF2H7, CREBH, and CREB4 (reviewed in [26]). However, despite their structural similarities, each ATF6 homolog seems to have specific functions in UPR regulated processes in specific organs and tissues [26].

2.1. β Cells Compensation and UPR Actors. β cell is a highly specialized secretory cell which responds to elevated postprandial glycaemia by increasing mRNA proinsulin translation and insulin secretion [27]. The periodic waves of proinsulin mRNA translation generate biosynthetic loads that

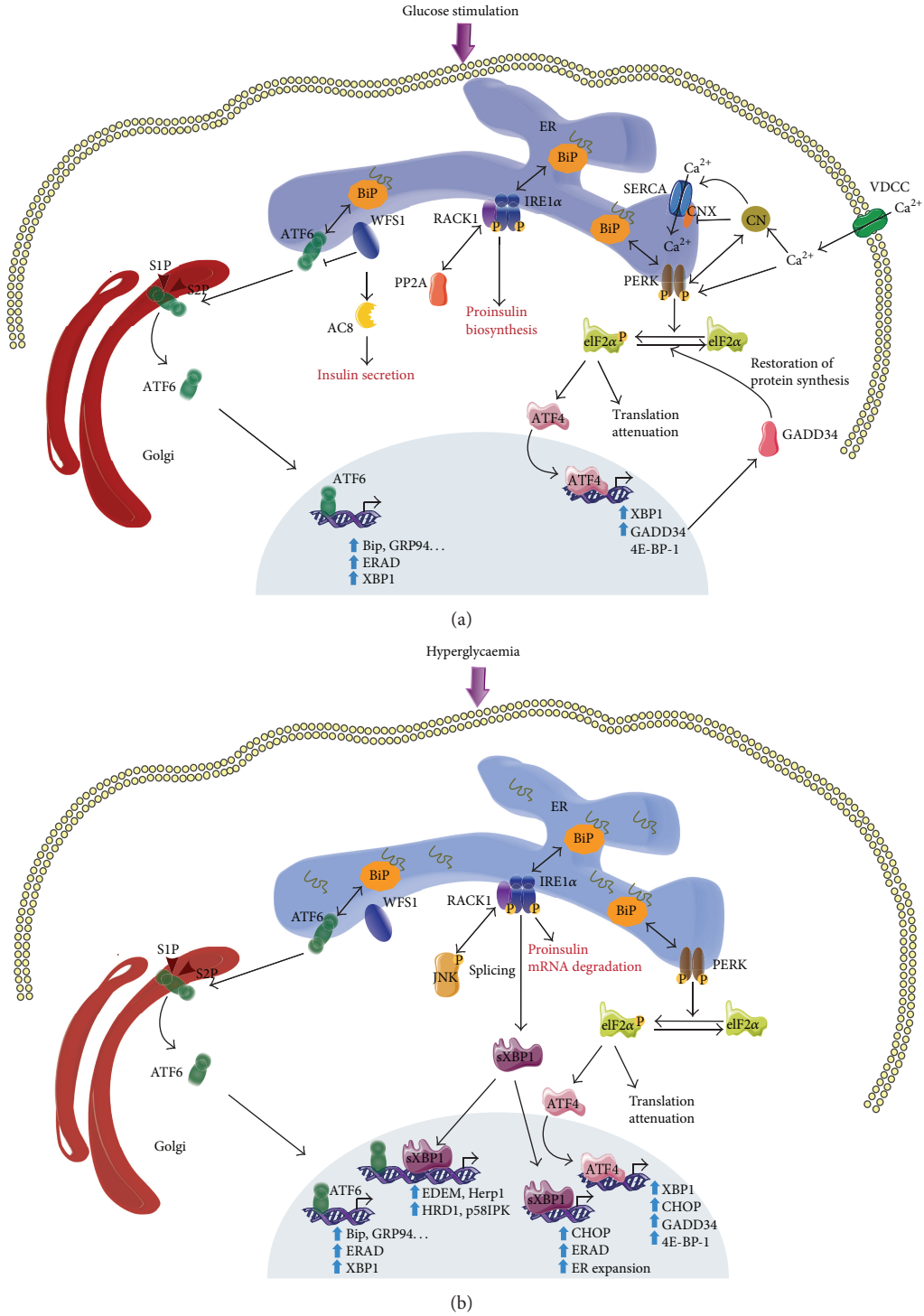

Figure 1: Physiological and physiopathological UPR activated pathways in β cells. (a) Under physiological conditions, increased proinsulin synthesis in response to postprandial glucose activates UPR to reduce ER stress and to promote β cell adaptation. The UPR triggers transcription of folding protein (BiP, GRP94,...), protein quality control (ERAD), UPR retrocontrol protein (GADD34), and attenuates protein translation (eIF2α). Additionally, the UPR regulates calcium homeostasis via PERK, promotes proinsulin synthesis via IRE1α, and increases insulin secretion via a WFS1-AC8 pathway. (b) Under physiopathological conditions, the UPR is hyperactivated leading to IRE1α hyperphosphorylation, which in turn induces proinsulin mRNA degradation, JNK pathway activation, and XBP1 mRNA splicing. XBP1s alone or in synergy with ATF6 lead to expression of ER chaperon (Herp1, EDEM, HRD1, p58IPK, and ERAD) and subsequent ER expansion. Both ATF4 and sXBP1 increase CHOP mRNA expression. Under these conditions the UPR feedback is deregulated.

induce UPR. To manage this burden imposed by proinsulin synthesis, β cells increase their ER size, as exemplified during diabetes development. In the prediabetic state, β cell must adapt its ER machinery to the new hyperglycaemic environment, promoting β cell compensation. The use of genetically modified mouse models and genetic studies from human diabetic patients demonstrated that UPR actors support this adaptation as well as β cell compensatory mechanism [4, 28].

2.2. Insulin Mutants and β Cells Compensation. Misfolding of proinsulin is associated with ER stress and severe dysfunctions leading to a massive destruction of β cells. *In vivo* evidences were observed in both Akita and Munich mice carrying cysteine residues mutations that interfere with disulphide bond formation [29, 30]. Interestingly, inactivation of the UPR induced proapoptotic Chop gene delayed the onset of diabetes in heterozygous Akita mice, suggesting a key role for CHOP in ER stress-mediated β cell apoptosis [30]. Diabetes of youth (MIDY) is a syndrome caused by a heterozygous mutation of the coding sequence of proinsulin leading to an autosomal-dominant and insulin-deficient diabetes [31]. This mutation has been shown to be the second most common cause of permanent neonatal diabetes related to ER stress [32, 33]. In line with these observations, inducible expression of the human analogue proinsulin C96Y mutation of Akita mice in rat insulinoma-1 (INS-1) caused ER stress and cell apoptosis. However, upregulation of UPR and ERAD seems to have a protective effect [34]. *In vivo* expression of the same proinsulin mutant driven by the weak Ins1 promoter induced both ER stress and pancreatic compensation [35]. Altogether these data demonstrate a clear link between misfolding of proinsulin and ER stress induction.

2.3. PERK-elF2α-ATF4 Pathway in β Cells Compensation. Loss of function mutation in the EIF2AK3 gene-encoding PERK was associated with WRS, which has been confirmed by the functional characterisation of *Perk* knockout mice [36]. Embryonic development of these mice is normal but they exhibit a postnatal growth retardation, skeletal dysplasia, and progressive loss of β cells, associated with defects in ER secretory machinery and proinsulin folding [37, 38]. However, generation of β cell specific Perk knockout mice revealed that β cell death was not increased, but rather β cell proliferation and differentiation were repressed during the embryonic and postnatal state [39]. Other studies on these mice models demonstrated an impaired ER to Golgi anterograde trafficking, retrotranslocation out of the ER, and proteasomal degradation, showing requirement of PERK for ER and Golgi integrity and processing of ATF6 [40]. In contrast to the first rapport, specific β cell inducible *Perk* deletion in mice showed a rapid progression of insulin dependent diabetes regardless of mice age [41]. The authors showed, on the one hand, that this phenotype was due to an increased β cell proliferation after the induction of PERK deletion due to the increased activation cyclin D-dependent kinase activity. On the other hand, they showed a significant increase in β cell death, associated with an activation of other UPR actors and a disturbance in calcium homeostasis [41]. Moreover, a recent work demonstrated that PERK, in concert with calcineurin, regulates ER calcium reuptake through calnexin interaction and a negative regulation of the sarcoplasmic endoplasmic reticulum calcium ATPase pump (SERCA) [42] (Figure 1(a)). PERK thus appears to sense glucose by direct sensing of ER calcium levels, raising the possibility that the primary function of PERK in β cell is to modulate proinsulin quality control and trafficking.

Within the β cell, phosphorylation of elF2α is mostly PERK dependent in ER stress conditions (Figure 1(b)). Mice harbouring a homozygote knock-in mutation at the PERK-mediated phosphorylation site (Ser51Ala) of elF2α display a severe β cell deficiency detectable in late stage embryos and die within 18 h after birth as a consequence of hyperglycaemia associated with defective neoglucogenesis [43]. Heterozygote mice development is normal; however, when challenged with a high fat diet, they develop severe obesity, glucose intolerance, and impaired insulin release. In this genetic context reduced insulin content, fewer granules, ER distension, and a prolonged association of proinsulin with BiP have been observed. Thus, elF2α phosphorylation is required for UPR to prevent β cell failure when insulin demand is increased. These findings demonstrate a central role of elF2α phosphorylation in β cell adaptation during compensation [44]. The generation of conditional homozygote ser51Ala elF2α mutation in β cell confirmed this observation. These mice exhibit a high rate of β cell apoptosis, likely caused by hypoinsulinemia, severe glucose intolerance, and hyperglycaemia. Furthermore, the authors showed ER distension and mitochondrial damage associated with a lower basal expression of the majority of UPR genes and β cell antioxidant responsive genes. Altogether these data indicate that the correct UPR and antioxidant response controlled by PERK-elF2α signalling are required for β cell adaptation and survival [45].

Phosphorylation of elF2α leads to attenuate global translation of most mRNA although translation of ATF4 is selectively stimulated in this context [11] (Figure 1(b)). The role of ATF4 in insulin secretion and β cell survival is controversial. Pioneer studies showed that *Atf4* knockout mice on a 129SV genetic background are lean, hypoglycaemic, and resistant to diet-induced obesity, probably as a result of increased energy expenditure. However, no effect was observed on plasma insulin level when mice were fed with normal diet, whereas insulin levels were shown to be lower in *Atf4* null mice compared to wild-type mice when fed with a high fat diet [46, 47]. Paradoxically, other studies using *Atf4* KO mice on C57BL6/J genetic background demonstrated that these mice are hypoglycaemic and hyperinsulinemic with an increased β cell mass and function. The phenotype of these mice could be secondary to an overexpression of osteocalcin in osteoblasts, a bone derived secreted molecule promoting insulin secretion and insulin sensitivity [48]. The genetic background may explain the differences observed in these Atf4 null mice models. The role of ATF4 in insulin synthesis and β cell adaptation to ER stress remains unclear, and further studies using a β cell specific *Atf4* KO mouse model may be useful to answer this question.

ATF4 activation by elF2α also leads to the transcription of elF4-E binding protein (4E-BP-1), a well-documented gene

involved in β cells adaptation to stress [49]. In fact, translation attenuation by elF2α phosphorylation is transient, subsequently leading to the feedback dephosphorylation by GADD34, whereas 4E-BP1 suppresses prolonged translation by the inhibition of cap-dependent translation [49, 50]. β cell specific 4e-bp1 KO mice are normal without any metabolic disorder when fed normal diet but are insulin resistant and show β cell defects under high fat diet due to induced ER stress [49, 51, 52]. Moreover, the inactivation of 4e-bp1 gene in Min6 cell line results in sensitization to ER stress and increased β cell loss and hyperglycaemia in diabetic mouse models [49]. These findings suggest a central role of 4E-BP1 in β cell adaptation to ER stress. In contrast, other groups indicated that suppression of 4E-BP1 expression is involved in beneficial effects of high-density lipoproteins on β cells survival [53], suggesting that the role of ATF4 in β cell compensation might depend on several cellular interactions.

2.4. IRE1α-XBP1 Pathways in β Cells Compensation. IRE1α is the major isoform expressed in the pancreas and plays a central role in β cell adaptation to ER stress (Figure 1(b)). IRE1α is required for embryonic development as demonstrated by the embryonic lethality of global IRE1α KO mice. IRE1α plays a crucial role in insulin biosynthesis. The generation of IRE1α conditional KO mice revealed that IRE1α deletion caused mild hyperinsulinemia and hyperglycaemia and a lower body mass under normal diet [54]. Physiological activation of UPR by glucose results in IRE1α phosphorylation, without increasing XBP1 mRNA splicing. Moreover, knockdown of IRE1α in INS-1 insulinoma cell line resulted in decreased proinsulin biosynthesis or insulin content without impacting global protein synthesis or insulin secretion, suggesting a beneficial effect of IRE1α activation by transit exposure to glucose in β cells [55]. However, chronic exposure to high glucose leads to hyperphosphorylation of IRE1α, which in turn results in selective degradation of proinsulin mRNA [56]. This may be part of the β cell protective mechanism from apoptosis under chronic hyperglycaemia induced ER stress. This adaptative mechanism combined to UPR activation may explain the reduced insulin secretion in type 2 diabetic patients in the absence of β cell death. IRE1α dephosphorylation is mediated by proteins phosphatase A2 (PP2A) through ternary complex containing the scaffold protein RACK1 (receptor for activated C kinase 1). Under glucose stimulation or ER stress, RACK1 mediates IRE1α, RACK1, and PP2A complex formation and promotes IRE1α dephosphorylation by PP2A, thereby inhibiting IRE1α activation and attenuating IRE1α-dependent increase in insulin production. Moreover, IRE1α activation is increased and RACK1 abundance is decreased in db/db mice [57]. The endoplasmic activity of IRE1α is also involved in the activation of a key metabolic enzyme, AMP-activated kinase (AMPK), in response to nitric oxide (NO) and ER stress in β cells [58]. AMPK is a holoenzyme activated by changes in AMP/ATP ratio, shifting from glucose to the use of lipids as an energy source in order to respond to cellular demand [59]. Activated AMPK by GTPase dynamin related protein 1 (DRP1) phosphorylation prevents ER and mitochondrial alteration in stressed β cells [60]. In addition IRE1α modulates nuclear factor κ light chain enhancer (NF-κB) target gene expression and IL-1β activation under mild ER stress, which could contribute to chemokine-induced β cell death [61].

Upon UPR mediating IRE1α activation, XBP1 splicing is the major event. Several reports indicated that XPB1s target genes and its downstream effect are cell specific and might be dependent on the activating pathways. Like IRE1α, XBP1 deficient mice died between 10.5 and 14.5 day after birth because of cardiac myocyte defects [62]. Heterozygous Xbp1 mice exhibited significant increase in body mass associated with a progressive hyperinsulinemia and glucose intolerance when fed with a high fat diet [63]. These mice showed increased ER stress and decreased insulin receptor expression in the liver. The β cell-specific deletion of XBP1 in mice resulted in a modest hyperglycaemia and glucose intolerance caused by decreased insulin secretion [64]. The loss of XBP1 markedly decreased the number of insulin granules and impaired proinsulin processing. Further analysis revealed that XBP1 deficiency not only participated in the ER stress in β cells but also caused constitutive hyperactivation of its upstream activator, IRE1α, which could degrade a subset of mRNAs encoding proinsulin-processing enzymes [64]. In summary, β cell defects in XBP1 mutant mice result from a combined effect of XBP1 suppression on canonical UPR and its negative feedback activation of IRE1α. Altogether these findings suggest a dual and opposite role for IRE1α in β cells. A precise regulated feedback circuit involving IRE1α and its product XBP1s is required to achieve optimal insulin secretion and glucose control. In contrast, sustain production of XBP1s leads to inhibition of PDX1 and MAFA expressions, promoting β cells dysfunction and apoptosis [54].

2.5. ATF6 Pathways in β Cell Compensation. Both ATF6 isoforms are required for positive regulation of UPR. However, the transcriptional activity of ATF6β is lower than that of ATF6α. The double knockdown of the two isoforms caused an embryonic lethality demonstrating overlapping functions of ATF6α and ATF6β, which are essential for embryonic development [24]. ATF6α KO mice demonstrated a severe hypoglycaemia suggesting that suppression of ATF6α increased insulin sensitivity [65]. Treatment of these mice with a pharmacological ER stress inducer leads to liver dysfunction and steatosis [66]. Furthermore, when fed with a high fat diet, ATF6α null mice developed insulin resistance associated with impaired insulin secretion and lower insulin content, reinforcing the idea of a key role of ATF6α in β cells adaptation and insulin resistance [65]. Recently, a basal expression of active ATF6α was demonstrated to be essential for β cell survival even under unstressed conditions. Interestingly, specific functions of ATF6α have been revealed depending on its interaction with XBP1. When ATF6α is acting alone, it induces the expression of a cluster of genes involved in protein folding such as BiP and GRP94. When it heterodimerizes with XBP-1 they are modulating the expression of specific class of target genes, such as genes involved in protein degradation (EDEM, Herpude1, HRD1, and p58IPK) [24]. In contrast, a deleterious effect of active ATF6α overexpression on

β cell function and expression of insulin, PDX1, and MAFA in INS-1 cells was shown [67]. Interestingly, some reports demonstrated that some ATF6 variants are associated with type 2 diabetes and new onset diabetes after transplantation (NODAT), suggesting potential links between ATF6α and human diabetes pathophysiology [68, 69]. It is important to note that, from the myriad of ATF6 homolog described until now, only old astrocyte specifically induced substance (OASIS) was identified in β cell [70]. However, microarray analysis of INS-1 β cell line overexpressing the active form of OASIS showed its implication in extracellular matrix production and protein transport but not in the classical ER stress response [70].

A great interest has been focused on the ATF6α negative regulator WFS1 because of its association with the Wolfram syndrome, a rare genetic disorder [71, 72]. Loss of function mutation in the *wfs1* gene encoding wolframin protein caused neurodegenerative disorders characterised by juvenile onset diabetes mellitus, optic atrophy, and hearing impairment [73, 74]. WFS1 KO mice exhibit an activated ER stress especially in β cells, leading to β cell loss through impaired cell cycle progression and increased apoptosis [75]. Conditional WFS1 knockdown in β cell induced diabetes as a result of enhanced ER stress and apoptosis [76]. Moreover WFS1 is essential for glucose and glucagon-like peptide 1 (GLP1) stimulated AMP production and regulation of insulin biosynthesis and secretion [77]. Under glucose stimulation, WFS1 translocates from the ER to plasma membrane, where it stimulates cyclic adenosine monophosphate [73] synthesis through an interaction with adenylyl cyclase 8 (AC8), which subsequently promoted insulin secretion [77] (Figure 1(a)). A recent report using induced pluripotent stem (iPS) cells to create β cells from individuals with Wolfram syndrome confirmed these observations. In this study, WFS1 deficient β cells showed increased levels of UPR genes and decreased insulin content, leading to β cell dysfunctions as previously described in mouse models [78].

2.6. The UPR/ER Stress Induction of β Cell Apoptosis. As discussed above, the UPR regulates both survival and death effectors. It is now clear that the three unfolded protein sensors—IRE1a, PERK, and ATF6—influence the life-death decision. The inability of UPR outputs to restore homeostasis may generate continuous signalling from these sensors, tipping the balance in favour of apoptosis. The ER might actually serve as a site where apoptotic signals are generated and integrated to elicit the death response. ER stress leads to apoptosis by activating both mitochondrial dependent and independent pathways. Several stimuli have been linked to ER stress-induced apoptosis including hyperglycaemia, exposure to long-chain free fatty acids (e.g., palmitate) [79–81], hyperinsulinemia occurring in the prediabetic stage [82], glucose deprivation [83], islet amyloid polypeptide (IAPP) expression [84], and exposure to inflammatory cytokine [85]. Players involved in the cell death response include PERK/elF2α-dependent transcriptional induction of proapoptotic transcription factor CHOP which represses Bcl-2 [86], IRE1-mediated activation of ASK1/JNK [18], and cleavage and activation of procaspase 12 (caspase 4 in humans) [87, 88].

CHOP has retained a special attention as a central mediator of apoptosis. Its expression is low under physiological condition but is strongly induced upon ER stress [89]. The induction of CHOP is regulated by ATF4 [11, 90] and ATF6 [91–93] and its role in ER stress-induced apoptosis was demonstrated both *in vitro* and *in vivo* [94]. Mice lacking CHOP are protected from renal toxicity of the ER stressor tunicamycin, an inhibitor of glycosylation [94]. CHOP deletion promotes β cells survival in both genetic and diet-induced insulin resistant mice models [30, 92]. Pancreatic β cells are also sensitive to oxidative stress, but β cells from CHOP knockout mice are protected and maintain insulin secretion under oxidative stress [92, 95]. Moreover, islets from these mice showed resistance to NO, a chemical agent implicated in β cells disruption in type 1 diabetes [96]. In contrast, CHOP deficiency in a genetic background of nonobese diabetic mice (NOD-Chop−/−) did not affect the development of insulitis, diabetes, and β cells apoptosis [97]. Interestingly, CHOP knockout mice on a C57BL/6 background showed a different phenotype, with abdominal obesity and hepatic steatosis, while preserving normal glucose tolerance and insulin sensitivity [98].

Under ER stress CHOP positively regulates the expression of genes involved in apoptosis including GADD34 [50, 99], the ER oxidoreductin 1 α (ERO1α) [100], death receptor 5 (DR5) [101], and the pseudokinase tribbles related 3 (TRB3) [102]. As shown for CHOP deletion, genetic inactivation of these genes protected against β cell ER stress-induced apoptosis [100, 103–105]. Additionally, CHOP represses the expression of the antiapoptotic gene Bcl2 and enhances oxidant injury [106]. Finally, deletion of CHOP was reported to prevent the cytokine-mediated cleavage of caspase 9 and caspase 3 and subsequent β cells apoptosis by reducing cytokine-induced NF-κB activity and the expression of key NF-κB target genes involved in apoptosis and inflammation [107].

ER stress-mediated apoptosis can also be signalled through IRE1α dependent activation of JNK pathway [18]. IRE1α interacts with TBF receptor associated factor 2 (TRAF2) and ASK1 mediating JNK phosphorylation [18, 108]. The analysis of ASK1 deficient mice showed that ASK1 loss of function attenuated insulin resistance, cardiac inflammation and fibrosis, vascular endothelial dysfunction, and remodelling induced by diet-induced obesity [109]. Moreover, deletion of ASK1 in homozygous Akita mice protected β cells from ER induced apoptosis and delayed the onset of diabetes [110]. The IRE1α/TRAF2 complexes also contributes to ER stress-induced apoptosis by promoting the clustering of procaspase-12 and its activation by cleavage in response to ER stress [111]. In addition, the IRE1α/TRAF2 complex interacts with IKK, an inhibitor of NF-κB, mediating its activation and promoting cell apoptosis in response to ER stress [112, 113]. Finally, members of Bcl2 family including BAX, BAK, BIM, and PUMA have been reported to directly interact with IRE1α demonstrating a physical link between members of the core apoptotic pathway and the UPR [114, 115]. In contrast, IRE1α forms a stable protein complex with Bax inhibitor-1 (BI-1) protein, suppressing cell death [116]. The IRE1α/BI-1 association decreased the ribonuclease activity of IRE1α and seemed to be required for early adaptive responses against ER

stress-induced apoptosis [116]. The control of IRE1α activity appears to be central in the mechanism protecting β cells from ER stress-induced apoptosis. Further studies are needed to understand the various aspect of IRE1α regulation and the contribution of the others actors of UPR in the ER stress-induced apoptosis.

2.7. The UPR in Human Diabetes. Clear evidence of the existence of ER stress in human β cells has been reported in the last decade [2, 3, 6, 117]. First analysis of islets from human T2D patients showed an ER extension but modest signs of ER stress marker in human pancreatic samples and isolated islets. However, glucose stimulation induced increased UPR in T2D islets cells [3]. Some markers of ER stress are increased in T1D human islets with partial ER stress [117, 118]. A recent report shows an alteration in the expression of specific branches of UPR mediators in T2D β cells [6]. These findings support the hypothesis of a decline in β cell adaptation/compensation during the progression of diabetes in human.

3. Conclusion

The β cell has a marked capacity to adapt to environment changes by increasing its mass and function. Diabetic signs occur when this adaptative mechanism fails to compensate for the increasing insulin demand. Activation of UPR actors is triggered in the early stage of the compensatory mechanism and may play a central role in β cell adaptation and subsequent functions. Further studies are required to understand the physiological significance and the direct implication of ER stress and UPR in the early stage of diabetes physiopathology. Moreover, the relationships among UPR actors, their activation, and β cell fate (adaptation/survival versus β cell dysfunction/apoptosis) remain to be fully clarified. Theoretically, the size of β cell mass is controlled by a balance between proliferation and apoptosis. Either increase of β cell apoptosis or decrease in β cell adaptation and compensation could, therefore, reduce the β cell mass in T2D patients. Studies carried out during diabetes development are required to better understand the mechanism of compensatory capacity and subsequent β cell loss in humans. This is of particular interest, since it could have beneficial impact for the treatment of metabolic diseases such as diabetes.

It is important to note that most UPR molecules have an adaptive function in β cells. Their role in the switch from survival to apoptosis is clearly demonstrated *in vitro* and in animal models but it remains unclear whether the same mechanisms occur in human β cell. Isolating and culturing primary β cells may be very stressful and do not perfectly reflect the *in vivo* context. Therefore the use of alternative method such as immunohistochemistry is powerful to determine the role of each branch of UPR in diabetes.

Conflict of Interests

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

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Review Article

Role of MicroRNAs in Islet Beta-Cell Compensation and Failure during Diabetes

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Pancreatic beta-cell function and mass are markedly adaptive to compensate for the changes in insulin requirement observed during several situations such as pregnancy, obesity, glucocorticoids excess, or administration. This requires a beta-cell compensation which is achieved through a gain of beta-cell mass and function. Elucidating the physiological mechanisms that promote functional beta-cell mass expansion and that protect cells against death, is a key therapeutic target for diabetes. In this respect, several recent studies have emphasized the instrumental role of microRNAs in the control of beta-cell function. MicroRNAs are negative regulators of gene expression, and are pivotal for the control of beta-cell proliferation, function, and survival. On the one hand, changes in specific microRNA levels have been associated with beta-cell compensation and are triggered by hormones or bioactive peptides that promote beta-cell survival and function. Conversely, modifications in the expression of other specific microRNAs contribute to beta-cell dysfunction and death elicited by diabetogenic factors including, cytokines, chronic hyperlipidemia, hyperglycemia, and oxidized LDL. This review underlines the importance of targeting the microRNA network for future innovative therapies aiming at preventing the beta-cell decline in diabetes.

1. Introduction

The concentration of glucose in the blood is tightly monitored by the pancreatic islet beta-cell production of insulin. The main function of insulin is to reduce blood glucose levels by triggering the uptake and the storage of this carbohydrate by the cells of the body. The quantity of insulin released by beta-cells varies according not only to secretagogues such as glucose but also as a function of the insulin demand from target tissues. A feedback loop also exists between insulin sensitivity and insulin secretion, such that changes in sensitivity of peripheral tissues are balanced by corresponding increases in secretion, insuring preservation of euglycemia [1, 2]. A rise in the insulin demand occurs during normal body growth (from birth to early childhood periods), as a consequence of an increase in body weight and during pregnancy. To meet the requirement of insulin, beta-cells adapt both their mass and function to release sufficient insulin to maintain blood glucose homeostasis [1, 2]. Evidence for this compensatory process has been consistently provided by rodent models of obesity and diabetes and notably by the emerging availability of human pancreas necropsies [2, 3]. Indeed, beta-cell mass and function in pancreases of nondiabetic or prediabetic obese individuals is larger than in lean normoglycemic subjects [3–5]. In obesity beta-cell mass increases by 30–40% whereas insulin secretory output augments by 100% [6]. Conversely, diminished beta-cells mass and function contribute to the decrease in plasma insulin level in individuals with diabetes. Postmortem histology further a 20–65% decrement in beta-cell mass in islets from obese individuals with type 2 diabetes (T2D) when compared to BMI-matched nondiabetic subjects [3–5, 7–9]. This adaptive capacity of human islets to obesity has been confirmed in experimental murine models [10, 11]. In one study, human islets were

grafted in an immunodeficient mouse strain sensitive to high fat-diet (HFD-)induced obesity [10]. This mice model is used for longitudinal studies of islets exposed to an obesogenic environment [10]. Enlarged volume of human beta-cells was observed in xenotransplanted mice fed with HFD for 12 weeks [10]. However, despite the gain of beta-cell mass and the increase in insulin expression, these mice displayed hyperglycemia. This study confirms the requirement for an appropriate number of functional beta-cells to circumvent insulin resistance [10]. Therefore, insulin deficiency in T2D may in part result from an insufficient number of functional beta-cells under conditions such as ageing, weight gain, or metabolic alterations [7, 12, 13].

Despite intensive research, current treatments of T2D do not prevent the appearance of long-term complications and, over time, can also become inefficient to insure appropriate glycemic control. This inefficacy may result from the fact that available strategies do not permit to protect beta-cells against their inescapable decline. The existing therapies with exogenous insulin or hypoglycemic agents for type 1 diabetes (T1D) are also unsatisfactory, since they do not offer a cure and are mostly insufficient for preventing the secondary complications associated with diabetes [14]. Transplantation of a sufficient number of pancreatic beta-cells can normalize blood glucose levels and may prevent the complications of diabetes [15]. However, immunosuppressive therapy is a current obstacle in transplantation and beta-cells from cadaveric donors are in such a short supply that transplants can be provided only to a limited number of patients. Regeneration of the functional beta-cell mass in patients could potentially represent an alternative to transplantation. In view of the inefficacy of the current treatments and the increasing global prevalence of diabetes [16], it is urgent to intensify efforts for developing new therapeutic strategies for both T1D and T2D. In this regard, it is tempting to postulate that strategies aiming at improving beta-cell function and mass plasticity as well as beta-cell survival under proapoptotic conditions could be of major interest for designing innovative therapeutics to prevent beta-cell decline and restore their functional adaptive ability in diabetes.

Adaptive capacity of beta-cell mass and function depends on the activity of transcriptional and translational regulators, which tightly and timely modulate the expression of genes in response to environmental cues. The noncoding microRNAs (miRNAs) are extremely important to accomplish this task [17]. MiRNAs act as translational repressors that bind to the 3′UTR of target mRNAs, leading to translational inhibition and/or messenger degradation [18, 19]. Each miRNA can have hundreds of targets, thereby triggering pleiotropic effects in beta-cells. This review provides insights into the pivotal role of miRNAs in beta-cell adaptation and failure during diabetes [20–26].

2. miRNAs Required for Beta-Cell Specification Fate

The regulation of the beta-cell mass in adult life results from the dynamic balance between proliferation, neogenesis, and apoptosis. The mechanisms underlying the control of these phenomena are participating also to normal pancreas development, and thereby can help in understanding the compensatory mechanisms elicited in response to environmental cues and metabolic changes [27, 28]. The pancreas derives from a pool of endodermal cells. At the initial stage, the proliferation of the progenitor cells is stimulated by growth factors and other signalling molecules produced by the surrounding mesenchyme. This process is governed by a sequential cascade including the appearance of neurogenin3 (Neurog3) [29]. The number of Neurog3 expressing cells increases and peaks at embryonic day e15.5, after which the level of this transcription factor gradually declines [29]. Neurog3 is undetectable in insulin- and glucagon-producing cells, suggesting that it is not necessary for postnatal islet function [29]. In fact, transient expression of Neurog3 is critical for temporarily allowing the lineage-committed transcription factors required for the differentiation of the endocrine progenitor cells, which will give rise to the endocrine cell subtypes within the islets [29–31]. Ablation of Neurog3 prevents the generation of all pancreatic endocrine cells in mice. Evidence for a role of miRNAs in the control of Neurog3 during pancreas development has been provided by a mice model in which the pancreatic expression of the large majority of the RNAs has been abolished. miRNAs are usually generated by RNA polymerase II**.** This enzyme initially yields pre-miRNA molecules containing a hairpin loop, which undergoes sequential processing including cytosolic excision of the hairpin by the ribonuclease (RNase) type III Dicer1 [32]. In mammals, the loss of the RNase III domain of Dicer1 blocks the formation of most miRNAs [32]. The islet-specific *Dicer1* knockout mice generated using the *Pdx1*-Cre transgene survive until birth but fail to grow and die by P3 [20]. The pancreas of Dicer1-null mice displays an almost absolute loss of insulin-producing cells and there is a marked decrease in other cell types at e18.5 [20]. The defect of endocrine cells observed in the *Dicer1* knockout mice is associated with an increase in Hes1 level and a reduction in the formation of endocrine progenitor cells expressing Neurog3 [20]. Besides the induction of Notch signaling by Hes1, a possible synergistic mechanism accounting for Neurog3 inhibition during pancreas development could be a direct control by miRNAs. Demonstration of this hypothesis has been attempted in a model for pancreatic regeneration [33]. Regeneration of beta-cells following a 50 or 70% pancreatectomy is not associated with induction of Neurog3 protein in progenitor cells despite the presence of the transcript. This result prompted the authors to propose a posttranslational control of Neurog3 expression mediated by miRNAs [33, 34]. Results from global miRNA profiling in regenerating pancreas after partial pancreatectomy have highlighted upregulation of 4 miRNAs including miR-15a, miR-15b, miR-16, and miR-195 (Table 1) [33]. All the four miRNAs are predicted to target the Neurog3 mRNA, suggesting that they could contribute to the posttranslational regulation of the transcription factor [33]. Whether these miRNAs individually contribute to pancreas development has not yet been investigated.

Table 1: miRNAs required for beta cell specification fate and pancreas development.

miRNAs	Known functional effect	Targets	References
mir-15a, miR-15b, miR-16, and miR-195	Pancreas development, beta-cells fate and regeneration	Neurog3	[33]
miR-375	Beta- and alpha-cells expansion		[42]
miR-7a	Beta-cell proliferation	mTOR pathway components	[44, 45, 48]
miR-124a	Pancreas development	Foxa2	[33]

3. miRNAs Are Required for Proliferation of Progenitor Cells and Mature Beta-Cells

When progenitor cells start expressing insulin they stop dividing. However, the beta-cell mass continues to expand during fetal and postnatal growth [28, 35–37]. A process that could account for beta-cell mass expansion in rodent is replication. In normal rats, the beta-cell population approximately doubles each day starting from the 16th day after conception [36]. After birth the beta-cell population still grows but during adult life at a much slower pace [37–39]. A role for miRNAs in the control of differentiated beta-cells has been highlighted by the generation of a mice model with beta-cell specific ablation of Dicer1. Disruption of the enzyme using the rat insulin promoter 2 (RIP-)Cre transgene leads to alteration in islet morphology, reduction in beta-cell number, and impairment in glucose-induced insulin secretion [40, 41]. Marked perturbations in beta-cell expansion and mass have been reported in knockout mice for individual miRNAs. The first one for which a major role in pancreatic development has been demonstrated is miR-375 (Table 1) [22, 42]. This miRNA is highly enriched in human and mice beta-cells [22]. The importance of miR-375 in pancreatic endocrine cell development has emerged from studies in zebrafish embryos [43]. Injection of anti-miR-375 morpholinos into one- or two-stage embryos resulted in disruption of the islet cell phenotype [43]. The miR-375 KO mice have been instrumental for unveiling a role for this miRNA in beta-cell expansion besides its involvement in the control of glucose-induced insulin secretion [42]. A 30–40% decrease in beta-cell mass has been measured within islets from these mice and, strikingly, a 1.7-fold increase in alpha-cells [42]. The combined hyperglucagonemia and hypoinsulinemia in miR-375 KO animals led them to develop hyperglycemia [42]. Other miRNAs such as miR-7a have been shown to potentially contribute to beta-cell expansion during pancreatic organogenesis. miR-7a belongs to the evolutionarily conserved miR-7a/b family and is abundant in beta-cells of rodent and human islets [44]. Inhibition of miR-7a activates the mammalian target of rapamycin (mTOR, a.k.a FRAP, RAFT, or RAPT) pathway in the mouse MIN6 insulin-producing cells and in primary mouse islets [45]. Activation of this evolutionarily conserved serine/threonine protein kinase promotes beta-cell replication and expansion of the beta-cell mass [46, 47]. The mTOR pathway can be divided into two biochemically and functionally distinct multicomponent complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [48]. The two complexes are pivotal for the control of beta-cell mass although their downstream targets are distinct [48]. Disruption of miR-7a leads to upregulation of the downstream targets of mTORC1, p70S6 K, eukaryotic translation initiation factor 4E (eIF4E) and two MAPK-interacting kinases that phosphorylate eIF4E, as well as one of the essential TORC2 components, Mapkap1 [48]. Consequently, activation of the mTOR pathway caused by the suppression of miR-7a results in increased proliferation of beta-cells. So far, independent studies have shown that human beta-cells can proliferate within islets or under *in vitro* condition but the rate is extremely low [3, 49–53]. It is noteworthy that the authors have observed a nearly 30-fold increase in human beta-cell proliferation upon the reduction of miR-7a level [48].

4. miRNAs Regulating Nutrient-Induced Insulin Secretion and Insulin Production

The function of mature beta-cells is to release appropriate amounts of insulin in response to its main physiological stimulus, glucose, and to other secretagogues, including the incretin hormone glucagon like peptide-1 (GLP-1) and its mimetics [54]. In fact, GLP-1 potentiates glucose-induced insulin secretion by interacting with the GLP-1 receptor [54]. In human islet beta-cells, efficient incretin-stimulated insulin secretion relies on the recruitment of a highly coordinated subnetwork of beta-cells [55]. Activation of GLP-1R elevates cAMP levels, which in turn promote insulin secretion via both protein kinase A (PKA-)dependent and PKA-independent mechanisms [54]. A downstream long-term effect resulting from PKA activation consists of the modification of the expression of several genes [54, 56]. Such modulation is deemed to contribute to the plasticity of the secretory response to GLP-1 and glucose. The expression of miR-375 has been shown to be controlled by the activation of the cAMP/PKA pathway [57]. Indeed, incubation of rat insulin-producing cells with the GLP-1 mimetic exendin-4 leads to a decrease in miR-375 levels in a mechanism that involves PKA [57]. Reduction of miR-375 occurs also in response to glucose but in this case in a cAMP/PKA-independent manner [57, 58]. Decrease of miR-375 in the mouse insulin-producing MIN6 cells enhances insulin secretion, whereas overexpression of this miRNA hampers the ability of the cells to secrete in response to glucose [22]. One of the targets of miR-375 that accounts for glucose-induced insulin secretion is myotrophin (Table 2) [22]. Silencing of the latter mimics the effect of miR-375 on insulin secretion [22]. Thus, the drop of miR-375 caused by glucose and exendin-4 could be beneficial for insulin secretion by increasing the expression of myotrophin [22].

Table 2: miRNAs regulating nutrient-induced insulin secretion and insulin gene expression.

miRNAs	Known functional effect	Targets	References
miR-9	Insulin secretion	Onecut-2, Sirt1	[26]
miR-21	Insulin secretion	VAMP2, Rab3a	[93]
miR-29a, b	Insulin secretion	Mctl1	[71]
miR-30d	Insulin transcription		[63]
miR-34a	Insulin secretion	VAMP2, Rab3a	[93]
miR-96	Insulin secretion	Noc2	[24]
miR-124a	Insulin secretion	Rab27a, Noc2, MCT1	[24, 71]
miR-204	Insulin transcription	MafA	[65]
miR-375	Insulin transcription, insulin secretion	PDK1, myotrophin	[22, 58]

Another miRNA required for insulin secretion is miR-9 (Table 2). This miRNA is upregulated during differentiation of human embryonic stem cells and during the formation of cells of both neuronal and pancreatic lineages [21, 59]. Moreover, appropriate expression of this miRNA is required for mature beta-cell tasks and probably during development. Either overexpression or silencing of miR-9 is deleterious for the secretory capacity of beta-cells [26]. In fact, manipulation of miR-9 level impinges the expression of the Onecut2 (Oc2) transcription factor, which in turn hampers the content of the secretory machinery component Slp4 [26]. As consequence of the increased level of Slp4, beta-cells insulin secretion in response to secretagogues is impaired [26].

Slp4 belongs to the Rab GTPase effector family that includes also RIM2, MyRIP/Slac2c, and Noc2 [60]. In beta-cells, these effectors are associated with Rab3a and/or Rab27a and regulate the assembly of the SNARE proteins SNAP25, Syntaxin-1, and VAMP-2, thereby controlling insulin exocytosis [60]. The expression of SNAP25, Rab3a, Rab27, and Noc2 are regulated by miR-124a in mouse insulin-producing cells [24]. Overexpression of miR-124 increases SNAP25 and Rab3a levels but reduces those of Rab27 and Noc2 [24]. miR-96 is also expressed in beta-cells and controls the expression of Slp4 and Noc2 [24]. The level of Slp4 increases and this of Noc2 decreases in cells that overexpress miR-96 [24]. The key role of miR-124a and miR-96 in the control of the level of several critical components of machinery governing insulin secretion suggests a potential participation of these miRNAs in the terminal differentiation of beta-cells.

Mature beta-cells have the exclusive task to produce insulin. At normal glucose concentrations, insulin represents approximately 1/3 of the synthesized proteins [61]. However, this ratio can reach almost 1/2 at 7 mmol/L glucose [61]. The increase of insulin production occurring within the first minutes to hours upon glucose exposure is mostly achieved through enhanced protein synthesis and mRNA stabilization. In contrast, at later time points it is mainly due to transcriptional and posttranscriptional mechanisms [62]. The long-term control of insulin mRNA levels triggered by glucose contributes to the replenishment of the hormone content and is achieved through the activation of transcriptional regulators and miRNAs. Culture of insulin-producing cells at high glucose concentration affects the expression of more than hundred miRNAs, including miR-30d which is able to increase insulin gene expression [63]. Key transcription factors involved in glucose-mediated control of insulin expression have been described in detail elsewhere [64]. These factors include v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA), pancreatic and duodenal homeobox 1 (PDX1), and neurogenic differentiation 1 (NeuroD) [64]. MAFA abundance is under the control of miR-204. In diabetes, the beta-cell expression of this miRNA increases in response to the elevation of the cellular redox regulator thioredoxin-interacting protein. In turn miR-204 reduces the expression of insulin [65]. Regulation of insulin transcription during development involves additional transcription factors including the members of the Onecut (OC) family [66]. In view of these findings, we tested the role of Oc2 and indirectly miR-9 in the control of insulin production. We found that overexpression or silencing of miR-9 decreases insulin expression (Figure 1(a)), indicating that adequate levels of this miRNA are required for maintaining optimal insulin mRNA levels. In addition, elevated amounts of miR-9 reduce the activity of a luciferase reporter construct driven by the rat insulin promoter (Figure 1(b)), suggesting a role for miR-9 in the control of insulin gene expression. Inactivation of OC-2 using a dominant negative construct mimics the effect of miR-9 on insulin promoter (Figure 1(b)). Thus, besides regulating glucose-induced insulin secretion, miR-9 appears also to be crucial for maintaining insulin mRNA levels in a mechanism probably involving Oc2. Accumulation of insulin mRNA in response to glucose relies on nuclear translocation of PDX1. Glucose-induced nuclear import of the transcription factor is triggered by activation of the phosphatidylinositol 3-kinase (PI3K) pathway [67]. This signaling cascade results in the phosphorylation of protein kinase B by the 3-phosphoinositide-dependent kinase 1 (PDK1) [68]. Beta-cell specific knockout of PDK-1 leads to a reduction in islet cell mass and the development of overt diabetes [69]. PDK1 has been identified as a target of miR-375 [58]. Overexpression of miR-375 in rat insulin-producing INS-1E cells decreases the expression of PDK-1, leading to reduction of insulin mRNA level [58].

Numerous genes that are required for glucose-induced insulin secretion and cells survival are highly or selectively expressed in beta-cells [60]. In addition, proper control of glucose-induced insulin secretion involves the absence or the low expression of "disallowed genes" including those coding for lactate dehydrogenase A (Ldha) and monocarboxylate transporter-1 (Mct1) [70]. Overexpression of ldha in insulin secreting cells affects glucose-induced insulin secretion. Islets of individuals with diabetes display an increase in the expression of LDHA when compared to controls [70]. Beta-cells elevation of MCT1 in mice fosters pyruvate-stimulated insulin secretion, thus leading to hyperinsulinism during exercise [71]. The absence of MCT1 in beta-cells could hence prevent inappropriate insulin secretion elicited by pyruvate. A mechanism that accounts for repression of the "disallowed" MCT1 in beta-cells could involve some miRNAs. The *MCT1*

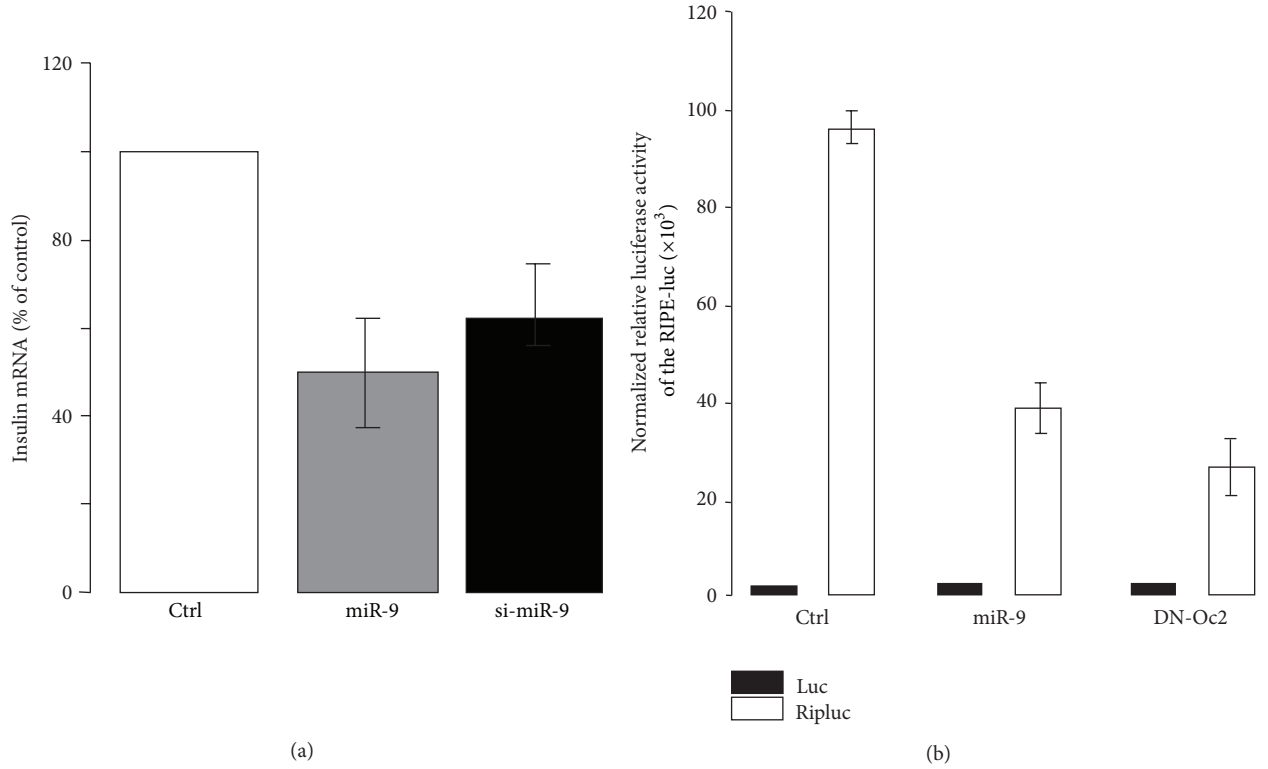

Figure 1: Role of miR-9 in insulin mRNA and promoter activity. (a) Effect of miR-9 on insulin mRNA. The RNA duplex containing the mature form of miR-9 [26] and a siRNA directed against miR-9 (si-miR-9) or a control oligonucleotide was transfected in MIN6 cells for 48 hrs. The expression of the preproinsulin mRNA was measured by quantitative PCR. The mRNA level was normalized against the housekeeping acidic ribosomal phosphoprotein P0 gene (*Rplp0*) and the expression level in cells transfected with the control siRNA was set to 100%. Data are the mean of ± SEM of 3 independent experiments. (b) Effects of miR-9 and dominant negative Oc2 mutant on the activity of an insulin reporter construct in MIN6 cells. MIN6 cells were transiently transfected with miR-9 RNA duplexes containing the mature form of miR-9 [26] or the dominant negative Oc2 mutant [26]. The cells were cotransfected with a luciferase reporter construct driven by a 600 bp fragment of the rat insulin promoter (Ripluc) and with pRLSV40, a construct producing a renilla luciferase activity under the control of the constitutive SV40 promoter. The firefly luciferase activity produced by Ripluc was normalized to the renilla luciferase activity to rule out differences in the transfection efficiency. The empty pGL3 basic (luc) was used as control. Each experiment was performed at least three times in triplicate.

mRNA is a direct target of miR-29a, miR-29b, and miR-124 [71]. From this example, it is possible that the miRNAs contribute to the silencing of "disallowed" genes in beta-cells.

5. miRNAs Associated with Compensatory Beta-Cell Mass Expansion in Pregnancy and Obesity

Pregnancy is the strongest physiological stimulus inducing beta-cell mass plasticity. The mass of beta-cells and their secretory activity returns to prepregnancy levels within the first 10 days following parturition in rodents [72, 73]. The levels of four miRNAs including miR-144, miR-218, miR-338-3p, and miR-451 are modified in islets from pregnant rats when compared to age-matched animals (Table 3) [74]. The expression of these miRNAs returned to resting levels after parturition [74]. *In vitro* experiments have confirmed a role for miR-338-3p and miR-451 in the control of beta-cell tasks [74]. While miR-338-3p levels are diminished, those of miR-451 are increased during pregnancy [74, 75]. Rodent and human beta-cell expansion during pregnancy is likely to occur thanks to enhanced proliferation combined with a minimal rate of apoptosis [72, 73, 76]. Overexpression of miR-451 does not increase the proliferation rate of insulin-producing cells but this miRNA protects the cells against apoptosis elicited by palmitate and cytokines [74]. Downregulation of miR-338-3p appears to be even more important in the adaptation of beta-cells during gestation. Indeed, reduction of miR-338-3p expression leads to a specific increase in proliferation of cultured insulin-producing islet cells and transplanted pseudoislets cells [74, 75]. Furthermore, as is the case for miR-451 overexpression, reduction of miR-338-3p protects the beta-cells against apoptosis evoked by diabetogenic conditions such as chronic exposure to elevated palmitate or cytokines, indicating that the decrease of miR-338-3p is pivotal for compensatory beta-cell mass expansion during pregnancy. Despite these proproliferative and antiapoptotic effects, miR-338-3p downregulation or miR-451 overexpression did not significantly impact insulin content and glucose-induced insulin secretion, indicating that upon changes in the level of these miRNAs the beta-cells retain a

Table 3: miRNAs associated with compensatory beta-cells.

miRNAs	Cell types/models	Expression change	Known functional effect	References
miR-132 miR-184	Islets of prediabetic db/db mice	Up Down	Beta-cells proliferation	[84]
miR-338-3p	Islets of pregnant rats and islets of prediabetic db/db mice and obese mice fed with a high fat diet Cells cultured with estradiol or incretins	Down	Beta-cells proliferation/antiapoptotic	[74]
miR-451	Islets of pregnant rats, islets of prediabetic db/db mice and obese mice fed with a high fat diet	Up	Antiapoptotic	[74]

fully differentiated phenotype. The beta-cell mass not only increases during pregnancy but also during insulin-resistance and obesity [1]. The gain of beta-cell mass compensates for the increased insulin demand from peripheral tissues, thereby maintaining euglycemia [1]. An increase of miR-451 and a decrease of miR-338-3p analogous to those observed in islets of pregnant rats are also detected in islets of obese mice fed with high fat diet [74]. Moreover, diminution of miR-338-3p occurs in young still normoglycemic but already obese *db/d*b mice, indicating a broader role for this miRNA in physiological islet adaptation [74]. The exact mechanisms governing the expression of miR-338-3p remain to be defined. During pregnancy, the level of estradiol and incretins such as GLP-1 is elevated [77, 78] and may be responsible for beta-cell proliferation [77, 79, 80]. GLP-1 is also increased in obese individuals possibly contributing to beta-cell mass expansion [77]. Interestingly, agonists of the GPR30 estradiol receptor and of the GLP-1 receptor are able to decrease miR-338-3p levels in beta-cells via a signalling cascade involving a rise in cAMP and the activation of PKA [74].

6. miRNAs Associated with Beta-Cell Dysfunction under Diabetogenic Condition

The compensatory processes described above precede beta-cell decline during the development of diabetes [2, 81]. Failure in mechanisms that maintain the adaptive capacity of islet beta-cells may account for impaired beta-cell function and mass. This hypothesis has been tested by measuring the expression of miRNAs in islets of leptin receptor deficient *db/db* mice of different ages. The *db/db* mice at 6 weeks of age are obese and insulin resistant [82, 83]. However, normoglycemia is preserved and manifestation of diabetes is delayed because of increased functional beta-cell mass. Besides the decreased expression of miR-338-3p [74], the adaptive islets of these mice display variations in other miRNAs (Table 4) [84]. These include an increase in miR-132 and a decrease in miR-184, miR-203, and miR-210 [84]. Overexpression of miR-132 and inactivation of miR-184 trigger proliferation in dispersed beta-cells from rat islets [84]. In contrast, *in vitro* reduction of miR-203 and miR-210 increases rat beta-cell apoptosis [84]. The reduction of miR-210 and miR-184 is more pronounced in isolated islets from overtly diabetic *db/db* mice, suggesting that an unbalance in the level of these miRNAs can result in a switch from beta-cell adaptation to programmed cell death [84]. In addition, changes in the expression of miR-199a-3p and miR-383 appear to contribute to beta-cell failure in diabetic *db/db* mice. Indeed, upregulation of miR-199a-3p and diminution in miR-383 increase rat beta-cell apoptosis *in vitro* [84]. At the present time, the miRNAs that are associated with compensatory human islets remain to be identified. So far, one study has quantified the miRNAs level in a small group of islets of individuals with and without type 2 diabetes [85]. Only an increase in the miR-187 level is associated with beta-cell failure in diabetes [85]. This result suggests that different miRNAs account for adaptation and decline of beta-cells in human and rodents during diabetes.

Chronic elevation in circulating levels of nonesterified free fatty acids (NEFAs) is associated with obesity and is an independent predictor of T2D development [86, 87]. Numerous studies have highlighted palmitate, the most abundant NEFA in blood, as a detrimental factor promoting insulin resistance and beta-cell dysfunction. *db/db* mice display an abnormally increased blood NEFA concentration [88]. Beta-cell failure elicited by this lipid includes a decrease of insulin expression, impaired secretory capacity in response to nutrients and/or loss of beta-cell mass via apoptosis [3, 4, 89]. Elevated palmitate levels are thought to synergize with chronic hyperglycemia in promoting beta-cell failure in obesity-associated diabetes [90]. The decrease in miR-184, miR-203 and miR-383 is mimicked by chronic exposure of beta-cells to palmitate and/or glucose, suggesting a role for glucolipotoxicity in the variation of these miRNAs observed in islets of diabetic mice [90]. Additional miRNAs are changed in *db/db* mice, probably contributing to beta-cell dysfunction and death [91]. The expression of miR-34a and miR-146 is indeed augmented in islets of these mice [91]. Elevation in their levels causes dysfunction and apoptosis and mimics the harmful effects of palmitate in cultured islets [91]. Palmitate also triggers beta-cell dysfunction by an indirect mechanism that involves activation of the inflammatory process [92]. Continuous infusion of palmitate in mice evokes an increase in M1-type proinflammatory monocyte/macrophages infiltration within islets [92]. Several studies contend a role for low grade inflammation as a major issue, which links obesity to the development of diabetes

Table 4: miRNAs associated with beta-cell failure.

miRNAs	Cells type/models	Expression change	Known functional effect	References
miR-21	Cells cultured with cytokines	Up	Glucose-induced insulin secretion and proapoptotic	[93]
miR-34a and miR-146a, b	Islets of diabetic db/db mice, cells cultured with cytokines or palmitate	Up	Glucose-induced insulin secretion and proapoptotic	[91, 93]
miR-184	Islets of diabetic db/db mice, cells cultured with glucolipotoxic condition	Down	Glucose-induced insulin secretion	[84, 90]
miR-187	Islets of individuals with type 2 diabetes	Up	Glucose-induced insulin secretion	[85]
miR-199a-3p	Islets of diabetic db/db mice	Up	Proapoptotic	[74]
miR-203 and miR-383	Islets of diabetic db/db mice, cells cultured with glucolipotoxic condition	Down	Proapoptotic	[90, 93]
miR-210	Islets of diabetic db/db mice	Down	Proapoptotic	[84]

Table 5: Global miRNA profiling of MIN6 cells cultured with human native and oxidized LDL with or without HDL. We compared by microarray analysis the expression of 350 miRNAs in MIN6 cells that were incubated with 2 mmol/L of human native (Na LDL) or oxidized LDL (oxLDL) cholesterol plus or minus 1 mmol/L of HDL for 72 hrs.

Microarray	Name	NaLDL	oxLDL	Change (log2)	Expression change	oxLDL	oxLDL + HDL	Change (log2)
NaLDL versus oxLDL	mmu-miR-9	1 622.36	739.3	−1.16	Down	2 417.08	3 407.71	0.46
	mmu-miR-21	6 073.84	15 447.81	1.37	Up	23 042.48	11 193.24	−1.01
	mmu-miR-98	7 909.01	2 817.75	−1.5	Down	12 432.59	16 691.57	0.42
	mmu-miR-192	222.59	514.66	1.02	Up	1 076.26	732.38	−0.51
	mmu-miR-325	1 018.38	462.41	−1.16	Down	1 757.68	2 642.59	0.65
	mmu-miR-342-3p	2 181.11	1 326.71	−0.73	Down	2 483.59	4 337.97	0.79
	mmu-miR-346	904.18	505.43	−0.85	Down	485.7	770.91	0.64
	mmu-miR-374	5 082.44	1 887.82	−1.43	Down	5 022.55	7 143.28	0.54
	Mmu-miR-708	366.85	712.44	0.94	up	825.11	402.52	−1.06
	mmu-miR-801	488.33	915.52	0.85	up	521.23	216.79	−1.29

[7, 89]. Interestingly, the levels of miR-34a and miR-146 are elevated by proinflammatory cytokines in isolated human islets and insulin-producing cells, indicating that the signaling cascades causing beta-cell failure elicited by palmitate and cytokines may converge and result in the activation of the same miRNAs [93]. Beside these two miRNAs, cytokines induce also the expression of miR-21 [93]. This miRNA plays a role in cell survival and can also affect glucose-induced insulin secretion by modulating the levels of components of the secretory machinery [93].

Besides chronic hyperlipidemia and hyperglycemia, patients with diabetes display an increased ratio of oxidized LDL over native LDL [94–96]. The concentration of oxidized LDL is already elevated in prediabetic individuals [97] and increases throughout the duration of the disease [98]. The rise of oxidized LDL is thought to result, in part, from the reduced antioxidant property of HDL [94, 98–100]. Elevation of oxidized LDL apparently correlates with reduction of plasma HDL concentration, a hallmark of metabolic syndrome [101]. Importantly, infusion of recombinant HDL in patients with T2D reduces glycemia [101]. The beneficial effect of HDL relies on both improved insulin secretion and glucose uptake in muscles [101]. Several independent groups, including ours, have confirmed the protective effect of HDL against the harmful effects evoked by oxidized LDL in beta-cells [94, 95, 101, 102]. Coincubation of islets and insulin-producing cells with HDL prevents the defective insulin production and glucose-induced insulin secretion observed in the presence of 2 mM oxidized human LDL cholesterol [94, 95, 101, 102]. Moreover, cell survival is strongly improved in the presence of HDL [94, 95, 101, 102]. Although native LDL above 3.1 mM cholesterol perturbs insulin secretion and cell survival [103, 104], at 2 mM cholesterol the lipoprotein does not affect the accomplishment of the tasks and the viability of beta-cells [94, 95, 101, 102]. A global microarray profiling was done to investigate the contribution of miRNAs in the adverse effects elicited by oxidized LDL. The modified lipoprotein modified the expression of a set of 10 miRNAs (Table 5). The expression changes were further prevented by coincubation with HDL (Table 5). However, quantitative PCR analysis confirmed the variation for only 5 of them (Figure 2). The expression of miR-9 was decreased, whereas that of miR-21 was increased in insulin-secreting cells cultured with oxidized LDL particles (Figure 2). As already mentioned, upregulation of miR-21 hampers glucose-induced insulin secretion by modifying the expression of components of the secretory machinery [26, 93]. Moreover, appropriate

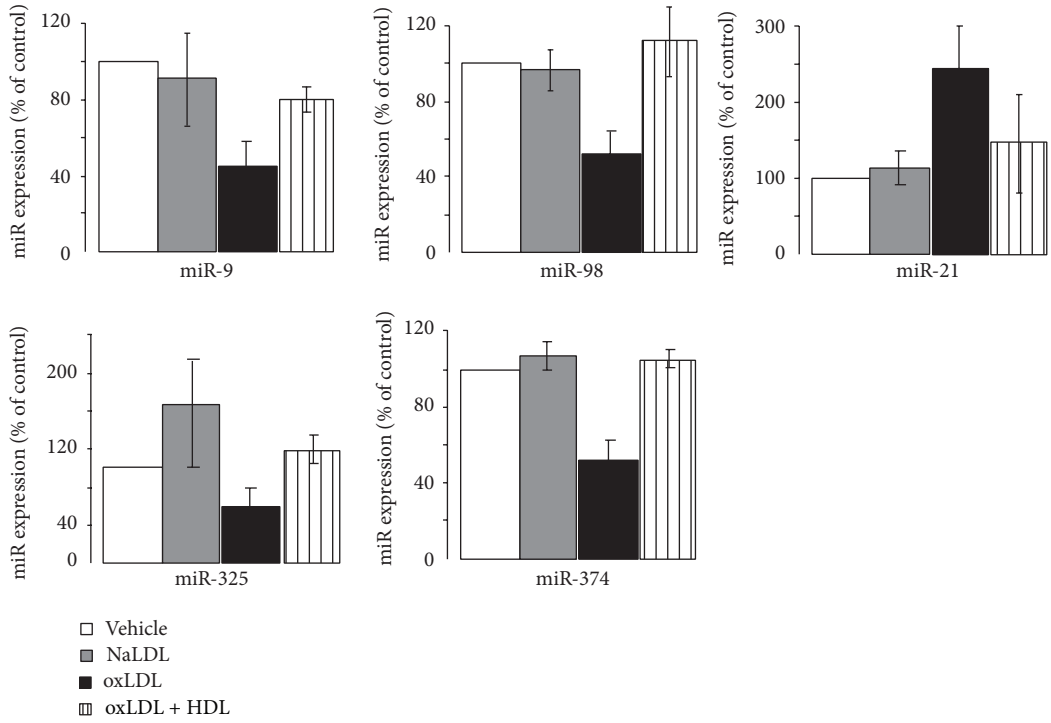

Figure 2: Identification of miRNAs differentially expressed in MIN6 cells cultured with human native and oxidized LDL. The expression of the indicated miRNAs was measured by quantitative RT-PCR in MIN6 cells that were cultured with vehicle, 2 mmol/L of oxidized LDL (oxLDL), and native LDL (NaLDL), plus or minus 1 mmol/L HDL-cholesterol for 72 hrs. Human plasma LDL and HDL fractions were isolated by sequential ultracentrifugation (LDL density, 1.063) as described [95]. Oxidation of LDL particles was done by incubation of 1 mg LDL protein/mL PBS with 5 μmol/L CuSO4 at 37°C for 6–8 h [95]. The oxidation reaction was verified as previously described by determining the lipid peroxide content [95]. The results are expressed as fold changes and correspond to the mean ± SD.

levels of miR-9 level are required to achieve optimal insulin expression (Figure 1(b)). Thus, the changes in these two miRNAs may contribute to beta-cell dysfunction provoked by the oxidized lipoprotein. Further studies will be needed to determine whether the decrease of miR-98, miR-325, and miR-374 (Figure 2) also contributes to the loss of specific beta-cell tasks and increased death caused by oxidized LDL.

It is widely accepted that the beta-cell decline in diabetes relies on genetic factors [105]. The contribution of genetic factors varies according to the forms of diabetes. In monogenic and dominant forms of diabetes, mutations in a single gene can lead to beta-cell failure and thereby to the development of the disease [105, 106]. The maturity-onset diabetes of the young (MODY) is a familial monogenic form of early-onset type 2 diabetes, which usually develops in childhood, adolescence, or young adulthood [105]. MODY is now classified in the group of "genetic defect in beta-cell function" with a subclassification according to the gene involved [105]. The most common mutation in the gene encoding transcription factor 1 (TCF-1)/hepatocyte nuclear factor 1a (HNF1A) that causes MODY3 is a frame shift mutation in exon 4, Pro291fsinsC-HNF1A [105–107]. The mutation within the gene results in a truncated protein that plays as a dominant negative action. Overexpression of this mutant in insulin-producing cells hampers glucose-induced insulin secretion [108]. Impaired insulin secretion caused by the mutated protein is associated with an elevation in the levels of miR-103 and miR-224. Thus, genetic variation may impact the expression of miRNAs, potentially synergizing with environmental stressors in triggering islet beta-cell dysfunction in diabetes [107].

7. Conclusion and Perspectives

miRNAs are essential regulators of beta-cell function as evidenced by the growing number of these small RNA molecules, which play a central role in normal development, plasticity, and dysfunction of insulin-secreting cells. Besides their intracellular function, a large set of miRNAs are released in stable form in body fluids including blood and urine. Variations in the blood miRNA pool are emerging as promising biomarkers of several diseases including diabetes [109]. Indeed, circulating miRNAs including miR-103 and miR-224 have been found in the blood of patients with diabetes [108]. Transport of miRNAs within blood is achieved through different pathways involving the association with HDL particles, exosomes, and other proteins such as argonaute 2 or nucleophosmin 1 [109, 110]. Defective beta-cells can release miRNAs into bloodstream following pathophysiological conditions. Future investigations should puzzle out the physiological meaning of these circulating RNAs and determine whether the pool of miRNAs released

in the blood differs according to the activation state of beta-cells. If so, monitoring these miRNAs would be insightful for monitoring whether beta-cells are in a compensatory or a failure condition. The extraordinary amount of new information provided by the discovery of the miRNAs has drawn researchers and clinical diabetologist to explore the potential involvement of another emerging class of non-coding RNAs, the long noncoding RNAs (lncRNAs) [111]. LncRNAs represent a heterogeneous population of RNA molecules longer than 200 nucleotides. The function of most of them remains unknown although several lncRNAs exert nonredundant roles in processes such as transcriptional regulation and survival [112–114]. A large number of lncRNAs is also present in human islets and some of them have their expression modified in diabetes [111]. There is no doubt that the coming years will witness the emergence of lncRNAs as additional players in the control of beta-cell function and/or in the regulation of lineage plasticity. The discovery of the regulatory potential of this emerging RNA world promises to unveil new opportunities for developing drugs capable of protecting beta-cells in the context of diabetes.

Conflict of Interests

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

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Clinical Study

Comparison of Metformin and Repaglinide Monotherapy in the Treatment of New Onset Type 2 Diabetes Mellitus in China

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Objective. This study was designed to compare the effects of metformin and repaglinide on the fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c) in newly diagnosed type 2 diabetes in China. *Methods*. A total of 107 newly diagnosed type 2 diabetic patients (46 women and 61 men) participated in the study. All patients received 3-month treatment of metformin or repaglinide. Fasting blood glucose and HbA1c were determined at baseline and at the end of the 3-month of treatment. *Results*. FPG and HbA1c decreased in both metformin and repaglinide groups after 3 months treatment ($P < 0.01$). The reduction of HbA1c was significantly greater in the repaglinide group ($P < 0.01$). Metformin decreases fasting insulin concentration and HOMA-IR ($P < 0.01$), and repaglinide improves HOMA-β ($P < 0.01$). Triglycerides (TG) were reduced in both groups ($P < 0.01$ in metformin group; $P < 0.05$ in repaglinide group), but total cholesterol (TC) and low-density lipoprotein (LDL) were decreased only after metformin treatment ($P < 0.05$). *Conclusions*. Both repaglinide and metformin were effective in glycaemic control in new onset patients with type 2 diabetes in China. Repaglinide had no effect on insulin sensitivity, but it improved β-cell function.

1. Introduction

With the rapid economic growth and changes of lifestyle in the past few years, diabetes has become a worldwide epidemic in the general population. The prevalence of diabetes is up to 11.6% according to the data released in 2013 by the China Noncommunicable Disease Surveillance Group [1]. It is followed by higher associated morbidity and mortality. Glucose disregulation is a major risk factor for cardiovascular disease [2], which has become the leading cause of death [3]. In fact, the management of blood glucose in patients with type 2 diabetes in China is far from ideal [4–6]. Less than 40% of all diagnosed diabetes cases were well controlled [1].

According to the AACE Comprehensive Diabetes Management Algorithm 2013, the entry HbA1c is below 7.5%; patients with new onset type 2 diabetes should be on monotherapy following the lifestyle modification [7]. Among all kinds of antidiabetic medications, metformin inhibits hepatic glucose output and increases peripheral glucose uptake and utilization. Due to its blood glucose-lowering efficiency, beneficial effects on body weight, and protective effect on the cardiovascular system [8], it is recommended as the first-line antihyperglycaemic management for type 2 diabetes [9–13]. It also benefits patients with diabetes in the improvement of insulin sensitivity. However, most effects of metformin originated from studies in Caucasian population. There are few data coming from Asian patients with type 2 diabetes.

In newly diagnosed Chinese patients with type 2 diabetes, the decreased insulin secretion, in particular the first phase of insulin secretion, is the main characteristic [14]. When the fasting glycaemia of the type 2 diabetic patients was above 7.0 mmol/L, the acute insulin release was substantially reduced [15]. Optimization of glycaemic control represents a major aim in the management of diabetes. Furthermore, it is important that strategies normalize glycaemia without increasing the risk of hypoglycaemia. Repaglinide belongs to the meglitinide class with benzoic acid in its structure [16]. It decreases blood glucose level by stimulating the release of insulin in a rapid-acting style. Therefore, repaglinide is one

of the most common antidiabetic medications in Chinese patients with higher postprandial glycaemia. Due to the complementary mechanism of metformin and repaglinide, the fixed dose of repaglinide and metformin has been used in the treatment of type 2 diabetes [17]. However, there were few studies comparing the effects of these two medications on glycaemic control in new onset Chinese patients with type 2 diabetes.

The study therefore aimed to compare the effects of metformin and repaglinide monotherapy on fasting blood glucose, HbA1c, body mass index (BMI), and lipids in the newly diagnosed Chinese patients with type 2 diabetes.

2. Methods

2.1. Subjects. All participants with type 2 diabetes, diagnosed by World Health Organization criteria, were recruited from the outpatient of the Endocrinology Department in Shanghai Renji Hospital. None had a history of coronary heart disease, abnormal renal function, active liver disease, chronic metabolic acidosis (including diabetic ketoacidosis), or severe chronic gastrointestinal disease. All of them were on two-week diet control before the enrollment.

The study protocol was approved by the Human Research Ethics Committee of the Shanghai Renji Hospital, and each patient was provided written informed consent. All studies were carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

2.2. Protocol (Table 1). All patients were given diet and lifestyle advice and asked to keep behaviour modification programme as a part of study. 107 patients were divided into two groups randomly. 54 patients (24 women, 30 men; age: 56.4 ± 1.6 years; BMI 26.15 ± 0.46 kg/m^2; HbA1c 6.95 ± 0.12%) received metformin 750–1500 mg/day, and 53 patients (22 women, 31 men; age 57.8 ± 1.3 years; BMI 24.45 ± 0.33 kg/m^2; HbA1c 7.42 ± 0.13%) received repaglinide 0.75–1.5 mg/day. The administration of metformin or repaglinide was adjusted according to the blood glucose levels in the first week (FPG higher than 7 mmol/L or postprandial glucose more than 10 mmol). During this phase, the doses of all patients were optimized and they were followed by a 3-month treatment period. Adverse events were evaluated according to the Medical Dictionary for Regulatory Activities (MedDRA version 14.0). If the patients had hyperlipidaemia (TC > 5.7 mmol/L or LDL > 3.4 mmol/L), they would be given lipid-lowering drugs (Liptor 20 mg). There were 11 patients in the metformin group and 4 patients in the repaglinide group taking lipid-lowering drugs.

All participants presented to the Endocrinology Department in Shanghai Renji Hospital on two occasions, at "baseline" and after 3-month treatment period. On the evening before each study day, patients consumed a meal before 7 pm. After the meal, patients fasted from solids and liquids (14 h for solids, 12 h for liquids) until the following morning. On the study day, patients attended the department at 8 am and an intravenous catheter was inserted for blood sampling. Blood samples were collected for measurement of the fasting blood glucose, HbA1c, and lipids. Body weight and height were measured at baseline and at the end of treatment. Compliance was monitored by assessing a checklist which the subject marked after taking the medication and checked by weekly telephone contact.

2.3. Measurements. Blood glucose concentrations were measured by the hexokinase method; plasma insulin concentrations were assayed by radioimmunoassay kit (Beijing Atom HighTech Co., Ltd., Beijing, China). The intra-assay coefficients of variation (CV) of insulin were 5.5%. HbA1c levels were measured by high pressure liquid chromatography; lipid profile parameters were determined on a clinical chemistry analyser (Roche Original Reagents, Stockholm, Sweden).

2.4. Statistical Analysis. Blood glucose and serum insulin concentrations were analyzed over fasting periods at baseline and after 3-month treatment. The data are reported as means ± standard error. Statistical comparisons were made using independent t-tests for values before and after 3-month treatment, and paired t-tests between groups with SPSS 16.0 and P values < 0.05 were considered significant.

3. Results

All patients completed the study and there were no adverse effects.

3.1. Fasting Blood Glucose Concentrations (Figure 1(a)). Fasting blood glucose was decreased after 3-month therapy both in the metformin (7.17 ± 0.15 mmol/L versus 6.29 ± 0.11 mmol/L, $P < 0.01$) and repaglinide groups (7.72 ± 0.17 mmol/L versus 6.46 ± 0.14 mmol/L, $P < 0.01$). There was no significant difference in reduction of fasting blood glucose between two groups.

3.2. HbA1c (Figure 1(b)). Significant improvements in HbA1c were seen in both metformin (6.95 ± 0.12% versus 6.34 ± 0.08%, $P < 0.01$) and repaglinide treatment groups (7.42 ± 0.13% versus 6.28 ± 0.09%, $P < 0.01$). HbA1c was decreased greater in the repaglinide group ($P < 0.01$).

3.3. Fasting Plasma Insulin (Figure 1(c)). In metformin group, fasting plasma insulin levels were significantly decreased from 11.95 ± 1.00 IU/L to 9.09 ± 0.74 IU/L after 3 months of treatment ($P < 0.01$). Fasting plasma insulin levels were 8.30 ± 0.53 IU/L at baseline and the values tended to be higher (9.64 ± 0.92 IU/L) after 3-month repaglinide treatment. Insulin resistance index (HOMA-IR) is fasting insulin (mU/mL) × fasting plasma glucose (mmol/L)/22.5. HOMA-IR is strongly related to insulin resistance. HOMA-IR decreased significantly during 3 weeks in metformin group (3.92 ± 0.38 versus 2.58 ± 0.22, $P < 0.01$), but it was not different after repaglinide treatment (2.92 ± 0.22 versus 2.86 ± 0.30, $P = 0.851$). Insulin sensitivity index (ISI) is 1/(FPG × FINS). ISI is useful for measuring insulin sensitivity. Similarly, ISI was reduced in metformin group (−4.29 ± 0.08 versus −3.88 ± 0.08, $P < 0.05$); but it was not different in

Table 1: The characteristic of 107 participants (54 in metformin group and 53 in repaglinide group) with type 2 diabetes, diagnosed according to World Health Organization criteria.

Parameters	Group metformin			Group repaglinide		
	Men	Women	Both	Men	Women	Both
Gender	30	24	54	31	22	53
Mean age	55.4 ± 2.2	57.5 ± 2.4	56.4 ± 1.6	56.9 ± 1.7	59.1 ± 2.1	57.8 ± 1.3
BMI	26.18 ± 0.57	26.11 ± 0.78	26.15 ± 0.46	24.80 ± 0.43	23.96 ± 0.50	24.45 ± 0.33
HbAlc	7.11 ± 0.18	6.76 ± 0.13	6.95 ± 0.12	7.38 ± 0.16	7.48 ± 0.22	7.42 ± 0.13
FPG	7.28 ± 0.23	7.04 ± 0.17	7.17 ± 0.15	7.72 ± 0.20	7.72 ± 0.31	7.72 ± 0.17
FPI	11.36 ± 1.08	12.69 ± 1.81	11.95 ± 1.00	8.17 ± 0.75	8.49 ± 0.73	8.30 ± 0.53
TC	5.38 ± 0.17	5.25 ± 0.18	5.32 ± 0.12	4.98 ± 0.16	5.75 ± 0.23	5.30 ± 0.14
TG	2.11 ± 0.20	1.77 ± 0.15	1.96 ± 0.13	1.97 ± 0.20	2.14 ± 0.26	2.04 ± 0.16
HDL	1.26 ± 0.10	1.28 ± 0.06	1.27 ± 0.06	1.23 ± 0.05	1.51 ± 0.15	1.35 ± 0.07
LDL	3.34 ± 0.13	3.28 ± 0.14	3.32 ± 0.09	3.18 ± 0.16	3.48 ± 0.24	3.30 ± 0.13

Data are shown by means and standard errors. It was only different in TC between men and women of repaglinide group ($P < 0.05$).

Table 2: Serum lipids are compared before and after metformin or repaglinide treatment.

Parameters	Before treatment	After treatment
	Group metformin	
TG (mmol/L)	1.96 ± 0.13	1.65 ± 0.11**
TC (mmol/L)	5.32 ± 0.12	5.02 ± 0.11*
HDL-C (mmol/L)	1.27 ± 0.06	1.26 ± 0.04
LDL-C (mmol/L)	3.32 ± 0.09	3.04 ± 0.09*
	Group repaglinide	
TG (mmol/L)	2.04 ± 0.16	1.79 ± 0.14*
TC (mmol/L)	5.30 ± 0.14	5.16 ± 0.13
HDL-C (mmol/L)	1.35 ± 0.07	1.29 ± 0.04
LDL-C (mmol/L)	3.30 ± 0.13	3.20 ± 0.11

Data are means and standard errors. *$P < 0.05$; **$P < 0.01$.

repaglinide group (−4.05 ± 0.07 versus −3.92 ± 0.10, $P = 0.159$). However, β-cell function was measured by HOMA-β. HOMA-β is an index of insulin secretory function derived from fasting plasma glucose and insulin concentrations: HOMA-β = FINS × 20/(FPG − 3.5). It was improved in repaglinide group (40.28 ± 2.41 versus 67.65 ± 6.08; $P < 0.05$), but the improvement was not found in metformin group (67.38 ± 5.03 versus 68.80 ± 5.84, $P = 0.81$).

3.4. BMI (Figure 1(d)). In metformin group, BMI was significantly decreased after 3 months of treatment (26.15 ± 0.46 kg/m^2 versus 25.52 ± 0.46 kg/m^2; $P < 0.05$). BMI was slightly decreased after treatment of repaglinide (24.45 ± 0.33 kg/m^2 and 24.30 ± 0.33 kg/m^2); but the reduction of BMI was not statistically significant.

3.5. Serum Lipids. Significant decreases in TG were found in both the metformin and the repaglinide groups during three months of treatment ($P < 0.01$ and $P < 0.05$ resp.). However, improvements in TC and LDL were only found in the metformin group ($P < 0.05$). No changes of HDL-C were observed in either treatment group (Table 2).

4. Discussion

We showed that both metformin and repaglinide significantly decreased fasting blood glucose and HbA1c in newly diagnosed type 2 diabetes in China. As the studies in western countries, metformin was associated with increased HOMA-IR and ISI, while repaglinide improved β-cell function. The antidiabetic effect of repaglinide was greater than metformin, but the improvement of TC and LDL was only observed in metformin group.

The United Kingdom Prospective Diabetes Study (UKPDS) has demonstrated good glycaemic control as evaluated by HbA1c, is related to the lower risk of micro- and probably macrovascular complications of diabetes [18–20]. The contribution of fasting and postprandial hyperglycaemia to different levels of HbA1c is various [21, 22]. The study indicated that postprandial blood glucose levels play a primary role in overall glycemic exposure with HbA1c being below 7.3% [21]. The fasting blood glucose concentrations are important only when HbA1c increased over 10.2% [21]. However, recent study demonstrated that, for those patients with type 2 diabetes who required intensive hyperglycemic control, the contribution of fasting blood glucose to overall hyperglycemic exposure was fairly high (around 78%) with HbA1c range from 7.0% [22]. Furthermore,

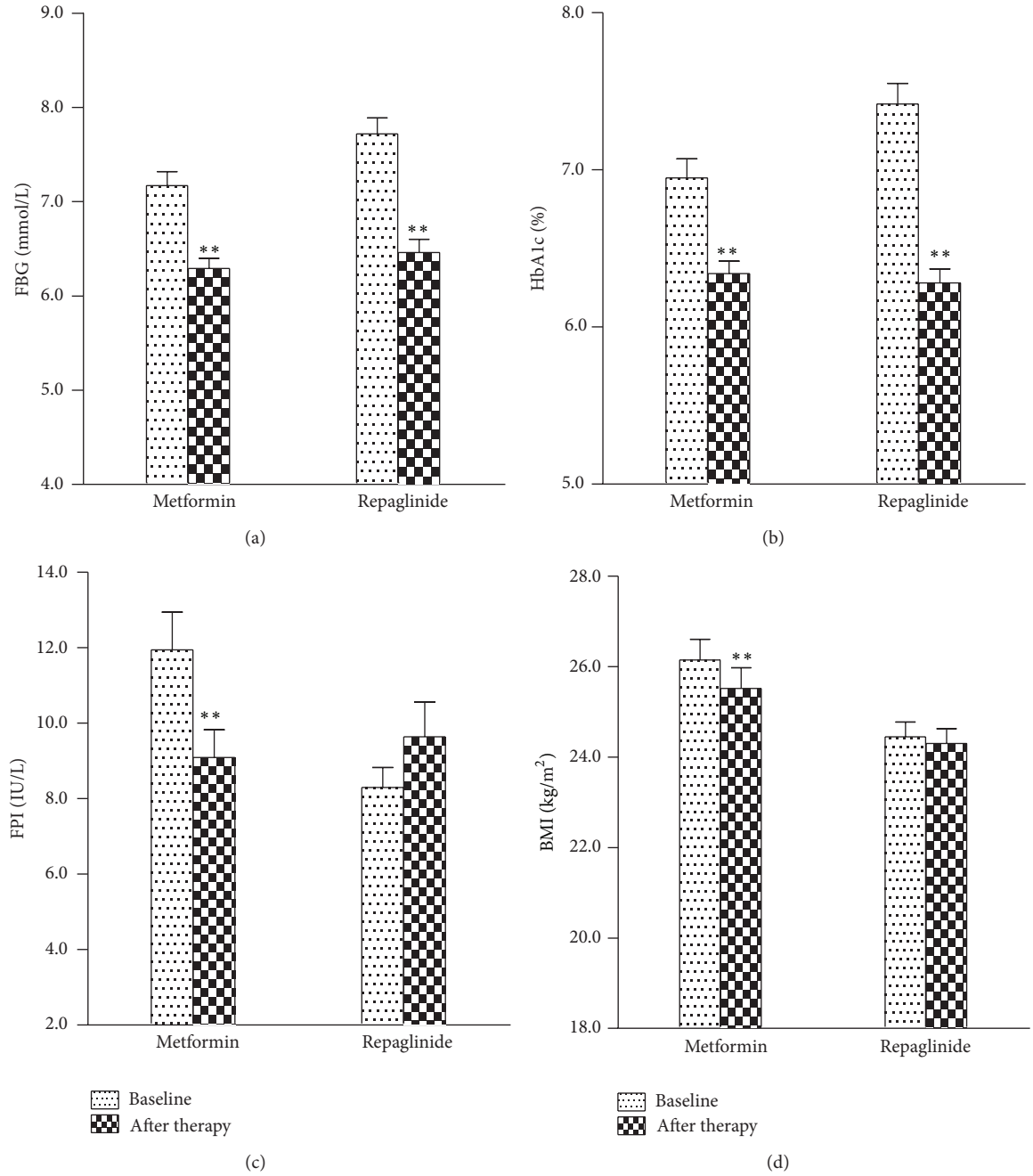

Figure 1: (a) Fasting blood glucose, (b) HbA1c, (c) fasting plasma insulin, and (d) BMI in 107 patients with type 2 diabetes before and after 3-month treatment of metformin or repaglinide. Data are means and standard errors. $^{*}P < 0.05$; $^{**}P < 0.01$.

fasting hyperglycaemia is more frequent than postprandial hyperglycaemia in the prevalence of diabetes in China [1]. Therefore, we evaluated the fasting blood glucose levels and HbA1c in therapy-naive patients with type 2 diabetes.

Compared to Caucasians, Asian type 2 diabetic patients are characterised by impaired β-cell function rather than insulin resistance [23]. Therefore, insulin secretagogues are still one of the most widely used antidiabetic medications in China. Repaglinide is mainly used as a medication to lower postprandial hyperglycaemia. It has been demonstrated that the effect of repaglinide on fasting blood glucose is similar to the sulfonylureas with less hypoglycaemia occurrence [24]. Metformin decreases gluconeogenesis and glycogenolysis in the liver to lower basal blood glucose. In the current study, both metformin and repaglinide were effective in FPG reduction in newly diagnosed type 2 patients. After 3 months of treatment, there was no statistic difference in decrease of FPG between repaglinide and metformin. However, HbA1c reduction with repaglinide was significantly greater compared with that with metformin. Although we did not measure the postprandial glucose, we would speculate that a more marked reduction in postprandial glucose after

repaglinide treatment at least partly contributes to the further decrease of HbA1c.

Repaglinide stimulates insulin secretion by closing ATP-dependent potassium channels and opening of calcium channels in the beta cell in response to ingestion. As a short-action insulin secretagogue, it mainly increases the first phase of insulin secretion, which is involved in suppression of hepatic glucose output and glucagon secretion [25]. It has been reported that repaglinide relieves the glucose toxicity to reduce fasting and postprandial insulin releases [26]. Thus, both HOMA-IR and HOMA-β were improved significantly during 12 weeks of treatment with repaglinide [27]. We confirmed that β-cell function has been improved in repaglinide group, but there was no improvement in HOMA-IR after repaglinide treatment. The different levels of fasting blood glucose should be considered. In our study, HbA1c and fasting blood glucose were comparably lower (7.7 ± 0.2 mmol/L) compared to the previous data in Italy (8.5 ± 1.3 mmol/L) [26]. Furthermore, we observed the insulinotropic effect in three months and the data might be changed on the long term. The parameters of β cell function were different with metformin. It is not surprising that metformin increases insulin sensitivity, expressed as HOMA-IR and ISI; but it has no effect in improving islet β cell function.

Lipidaemia represents an independent risk of cardiovascular diseases for patients with diabetes. The lowering effects of oral antihyperglycaemic medications on lipid profile and body weight should bring extra benefit for patients with type 2 diabetes. A study in China reported that repaglinide improves the lipid metabolism by reduction of TC in newly diagnosed patients with type 2 diabetes [28]. However, our study did not show any differences in TC or LDL after repaglinide dosage, although BMI and TG were decreased after 3 months. In conclusion, for overweight or obese type 2 diabetes patients, repaglinide can be considered as one of selecting antidiabetic medications. It is consistent with Chinese guideline for type 2 diabetes mellitus in 2010 [29].

This study has some limitations. Firstly, mean BMI of patients with type 2 diabetes in metformin group were higher than those in repaglinide group. However, BMI in both groups were in range of overweight (24.0–27.9 kg/m^2) in Asian population. Moreover, it has been reported that the effects of metformin on glycaemic response were similar among normal weight (BMI $<$ 24 kg/m^2), overweight, and obesity in Chinese patients with new onset type 2 diabetes [30]. Secondly, we did not measure postprandial blood glucose or plasma insulin concentrations after 3-month therapy just because repaglinide is well known for the prandial glucose regulation, but there was no study about the effects on fasting blood glucose in Chinese population. Thus, we focused on the effects of metformin and repaglinide therapy on fasting blood glucose in our study. Finally, the duration of this study was relatively short. Longer term studies are required in the future study.

In conclusion, this study demonstrated for the first time that both repaglinide and metformin monotherapies were effective in fasting blood glucose and HbA1c reduction, with the effect of repaglinide being greater in new onset Chinese patients with type 2 diabetes. Repaglinide improved β-cell function after 3-month treatment. However, the risk-predictive parameters of cardiovascular disease such as TC and LDL-C were only improved in metformin group.

Conflict of Interests

There is no conflict of interests to disclose.

Authors' Contribution

J. Ma and L. Y. Liu contributed equally to this work.

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Review Article

Decompensation of β-Cells in Diabetes: When Pancreatic β-Cells Are on ICE(R)

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Insulin production and secretion are temporally regulated. Keeping insulin secretion at rest after a rise of glucose prevents exhaustion and ultimately failure of β-cells. Among the mechanisms that reduce β-cell activity is the inducible cAMP early repressor (ICER). ICER is an immediate early gene, which is rapidly induced by the cyclic AMP (cAMP) signaling cascade. The seminal function of ICER is to negatively regulate the production and secretion of insulin by repressing the genes expression. This is part of adaptive response required for proper β-cells function in response to environmental factors. Inappropriate induction of ICER accounts for pancreatic β-cells dysfunction and ultimately death elicited by chronic hyperglycemia, fatty acids, and oxidized LDL. This review underlines the importance of balancing the negative regulation achieved by ICER for preserving β-cell function and survival in diabetes.

1. Introduction

The exposure of population to overfeeding and sedentary lifestyles has increased dramatically during the last decades worldwide. This has been accompanied by a rise in the incidence of obesity and therefore the associated morbidity and mortality. These complications are related to comorbid conditions including diabetes. Insulin resistance is the most common metabolic alteration related to obesity and is considered to be a critical link between adiposity and the risk for developing diabetes. However, in most of cases, obesity does not lead to diabetes. This situation is thought to result from the capacity of β-cells to compensate for insulin resistance by releasing appropriate amount of insulin in blood probably by an increased β-cells function and mass. When the cells decompensate and thereby fail to secrete adequate insulin in the face of increased hormone demand, then there, overt diabetes comes. In this respect, identification of leading mechanisms that account for β-cells compensation and decompensation would permit to pave the way for innovative therapeutic strategies of diabetes. The present review unveils a role for the cAMP pathway target inducible cAMP early repressor (ICER) as a central player for β-cells adaptation, which is impinged in β-cells under diabetes environmental stressors.

2. Portrait of ICER

ICER has been discovered as an inducible cAMP responsive element modulator (CREM) protein in neuroendocrine cells cultured with cAMP raising agents [1]. ICER is a small protein (<20 KDa), which contains one of the two CREM DNA-binding domains (DBDs) but without the activator and regulatory regions of the gene (Figure 1). CREM DBD I and DBD II are composed of a basic Leucine Zipper structure and have a strong homology with each other and with the unique DBD which is present on the CREB protein (Figure 1). Due to the presence of these two DBDs and to differential splicing, four ICER protein isoforms are possible. ICER I and ICER II isoforms contain, respectively, the DBD I and DBD II (Figure 1). These isoforms contain also the small exon γ of CREM gene, which instead can be missing in the two remaining isoforms: ICER Iγ and ICER IIγ. All four isoforms appear to be, in

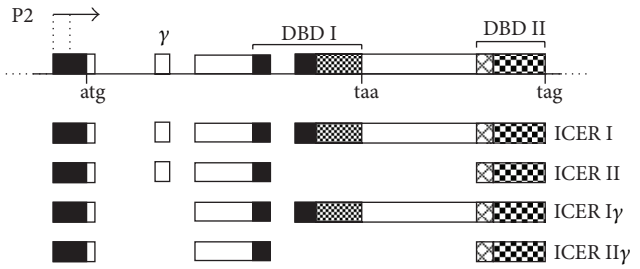

Figure 1: Expression of the ICER isoforms. ICER results from the P2 alternative promoter within the CREM gene. The ICER I and II isoforms are the results of alternative splicing. ICER Iγ and IIγ have the γ exon. DBD: DNA binding domains.

principle, functionally equivalent since they harbor one DBD. ICER binds cAMP response element (CRE) as homodimers and/or heterodimers with any member of the cAMP response element (CRE) binding protein/CRE modulator/activating factor 1 (CREB/CREM/ATF1) gene family [2–4]. Being composed of mainly the DBD, ICER can neither activate nor actively repress genes expression. However, when the expression level is high enough, it rather plays as a passive repressor by competing CREB/CREM/ATF1 transcriptional activators for binding to CRE (Figure 2). In mammalian cells there are thousands genes containing functional CRE [5, 6]. This implies that ICER is pivotal for regulation of genes expression in response to cAMP pathway.

ICER arises from the transcription of the *CREM* gene, directed via the P2 alternative internal intronic promoter [1]. The promoter contains a cluster of four CRE sites. Of note, ICER itself binds these sites and thereby represses its own promoter activity, in a negative feedback autoregulatory loop [7]. The kinetic of ICER induction is that of an immediate early gene, with transcript level peaking few hours after induction and thereafter rapidly declining. ICER is present in a wide array of different tissues such as nervous system, pituitary and pineal glands, thyroid, testis, liver, adipose tissue, pancreas, smooth muscles, skeletal muscle, cardiac muscle, bone, and cells of the immune system [8–17]. In the nervous system, especially in brain structures where a constitutive inhibition of cAMP-sensitive transcription seems to be necessary to maintain proper function, elevated basal level of ICER is required [18]. Notably, in the pineal gland, ICER is expressed in a circadian fashion, with high levels peaking during the subjective night followed by undetectable level in the subjective daylight [19]. This pattern of expression in the pineal gland is important for the transcriptional control of the rhythmic expression of arylalkylamine N-acetyltransferase, the rate-limiting enzyme controlling melatonin synthesis [20].

3. ICER as a "Brake" for Permitting Insulin Production and Secretion Return to Basal State

Insulin production, secretory function, and the rate of β-cells survival as well are regulated by the cAMP pathway. This is exemplified by the incretin Glucagon like peptide 1 (GLP-1), which triggers a rise of cAMP and the subsequent activation of CREB [21]. As mentioned above, thousands of genes can be regulated by CREB and ICER (some relevant targets for β-cells are presented on Figure 3). One of the direct targets of CREB is the neurogenic differentiation 1 transcription factor (NeuroD), which regulates the insulin expression and the sulfonylurea receptor 1 [22]. Among the other direct targets genes there are insulin itself and components of the exocytosis apparatus such as Rab3A and Rab27A, which are members of the Rab family, and two of their effectors, slp4 and Noc2 [23, 24]. The four genes contain a functional CRE able to bind ICER [25]. Overexpression of ICER in β-cells reduces the expression of the four secretory genes. These results have led to speculate that ICER is part of adaptive mechanism allowing the expression of the components of the secretory machinery to meet the insulin production [26]. After stimulation, insulin secretion returns to basal level. Induction of ICER could be a major mechanism permitting β-cells to reduce the secretory activity, while insulin expression is diminished. This β-cells activity is required to replenish insulin within ready releasable granules for the next meal or stimulatory conditions. Connexin 36 (Cx36) is a transmembrane protein that forms gap junctions for β-cells communication [27–31]. Cx36 function is required for the control of glucose-induced insulin secretion [31]. The gene coding for Cx36 contains a CRE and is negatively regulated by ICER [31], indicating that the control in the Cx36 level by ICER participates to the dynamic regulation of glucose-induced insulin secretion. Besides of regulating β-cell function, ICER could be instrumental for controlling β-cells survivals and death. In fact, β-cells overexpression of ICER in mice impinges β-cells mass by slowing proliferation [32]. Consequently, insulin secretion is collapsed and mice have developed diabetes. Direct decrease of Cyclin A expression by ICER accounts for decline in β-cells number in transgenic mice [33]. Insulin receptor substrate 2 (IRS-2) is required for β-cells proliferation and survival. IRS-2 is a target of CREB/ICER. Expression of a CREB dominant negative in β-cells provokes diminution of IRS-2 and activation AKT signaling, thus causing β-cell dysfunction and loss of β-cell mass [34]. The mitogen activated protein kinase (MAPK) 8 interacting protein 1 (*MAPK8IP1*) gene encodes islet brain 1 (IB1) also termed as JNK interacting protein 1, a protein that tethers kinases of the JNK pathway. The IB1 function is to preserve β-cells survival, insulin expression, and secretion in response to proapoptotic stimuli by regulating the c-jun N terminal kinases (JNK) pathway [16, 35]. *MAPK8IP1* contains within its proximal regulatory region several CRE. However, only one is capable to interact with CREB and to be negatively regulated by ICER [36]. Regulation of IB1 through this sequence is crucial for the protective effect of the GLP-1 mimetic exendin-4 [37]. The protective effect of IB1 is thought to involve JNK3 activation (Figure 3).

Pancreatic β-cells express the molecular clock proteins controlling circadian rhythm of insulin secretion and impairment of some member of the clock genes such as circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1), leading to hypoinsulinemia and

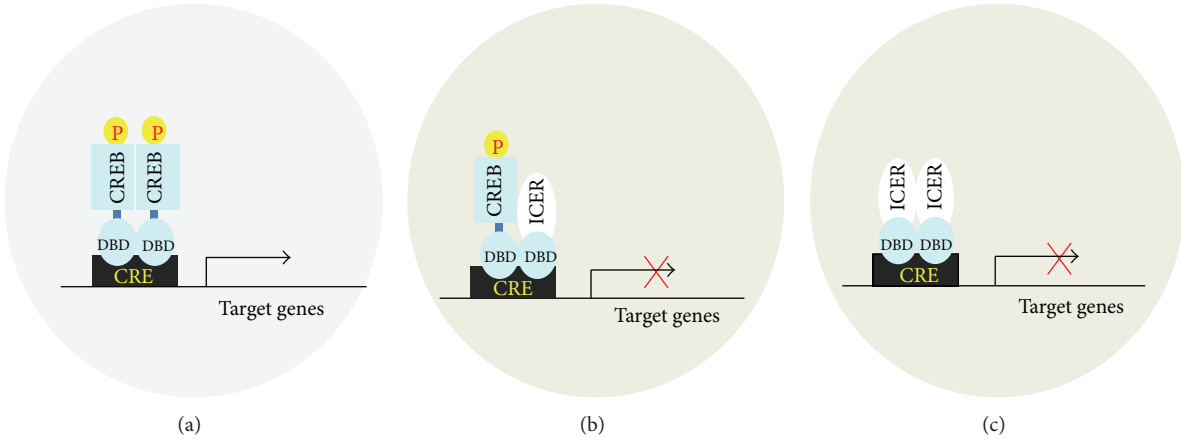

Figure 2: Schematic model for function of the passive repressor ICER. (a) Binding of CREB to CRE occurs when CREB is phosphorylated and the level of ICER is low. CREB can either homodimerize or form heterodimers with other activators, thereby activating gene expression. ICER competes with CREB for binding to CRE when it reaches a certain level. In this case, ICER can either (b) heterodimerize with CREB or (c) homodimerize.

diabetes [38–41]. CLOCK and BMAL1 work through interwoven positive and negative feedback loops [42]. The two proteins form heterodimers that activate transcription of the genes coding for Period (PER) and Cryptochrome (CRY). PER/CRY heterodimers form the negative limb, which in turn inhibits the activity of CLOCK/BMAL1, thereby generating circadian rhythms of transcription/translation. At the heart of the system is the ability of CLOCK/BMAL1 heterodimers to recognize and bind the E-Box elements which are present on the promoters of both *Per* and *Cry* genes. In addition to the E-Box, *Per1* and *Per2* genes contain functional CRE elements [39, 40]. Regulation of these genes by CREB is important for the fine tuning and modulation of clock genes in response to changing environmental cues. For instance, CREB-mediated upregulation of the *Per1* gene in the suprachiasmatic nucleus neurons is required for the photic resetting that takes place during the dark-light circadian transitions [43, 44]. Moreover, CREB plays another important role in the modulation of the molecular clocks in peripheral organs, especially in the liver where it is implicated in the control of gluconeogenesis [45, 46]. Interestingly, recent data show that ICER regulates the *Per1* gene in hepatic and adrenal gland clocks [47] and such regulation could account for circadian melatonin production in the pineal gland [19]. In view of these findings, it is possible that such a mechanism could take place in β-cells but this remains to be addressed.

4. Deregulation of ICER in Response to Environmental Stressors Associated with Diabetes

Typically, ICER activity results from a rise of its expression. Repression of target genes ensues when the expression of ICER reaches appropriate amount for competing CREB, CREM, and eventually ATF for binding to CRE (Figure 2). Such regulation could represent an adaptive mechanism for cells to return to their basal state after stimulation. In this respect, it is predictable that deregulation in the levels of ICER could strikingly perturb β-cells function and thereby glucose homeostasis. Several lines of evidence seem to argue in favor of such hypothesis. The first clue comes from a study carried out on Goto-Kakizaki (GK) rats, a well-characterized model of genetic nonobese type 2 diabetes in which β-cells function is impaired [48]. Isolated islets from these rats display high levels of CREM repressor including ICER I, indicating that the increase of ICER could contribute to β-cell dysfunction. Insulin secretion usually increases as the consequence of insulin resistance. However, glucose sensitivity of β-cells can fail to overcome insulin demand overtime. In this case overt diabetes appears. In islets of obese mice fed with a HFD, increase in the ICER level has been monitored [49]. Obesity is characterized by chronic elevation of nonesterified free fatty acids (NEFAs) including the saturated NEFA palmitate [50]. Chronic hyperglycemia resulting both from insulin resistance and glycemic excursion from the meal can also appear in obesity. There are clues that palmitate and chronic hyperglycemia may account for the increase of ICER in defective β-cells in obese animals. Prolonged elevation of palmitate and glucose, individually, hampers insulin secretion in human individuals and exerts harmful effects in β-cells. *In vitro* experiments have unveiled that increase in ICER is partly responsible of the adverse effects elicited by both diabetogenic factors.

Modification in the lipoproteins level is observed in obese individuals and is hallmark of metabolic syndrome. Increased levels of oxidized LDL-cholesterol particles together with a decrease in plasma concentration of HDL particles are seen at present as additional potential diabetogenic stressors, while they increase the risks of patients for developing cardiovascular diseases. Low plasma level of HDL and specific antibodies against oxidized LDL are found in patients with T2D. Perturbations in the two lipoproteins are further already observed in metabolic syndrome and they are worse throughout the duration of diabetes. Infusion of recombinant HDL in patients with T2D reduces glycemia by an increase in insulin secretion

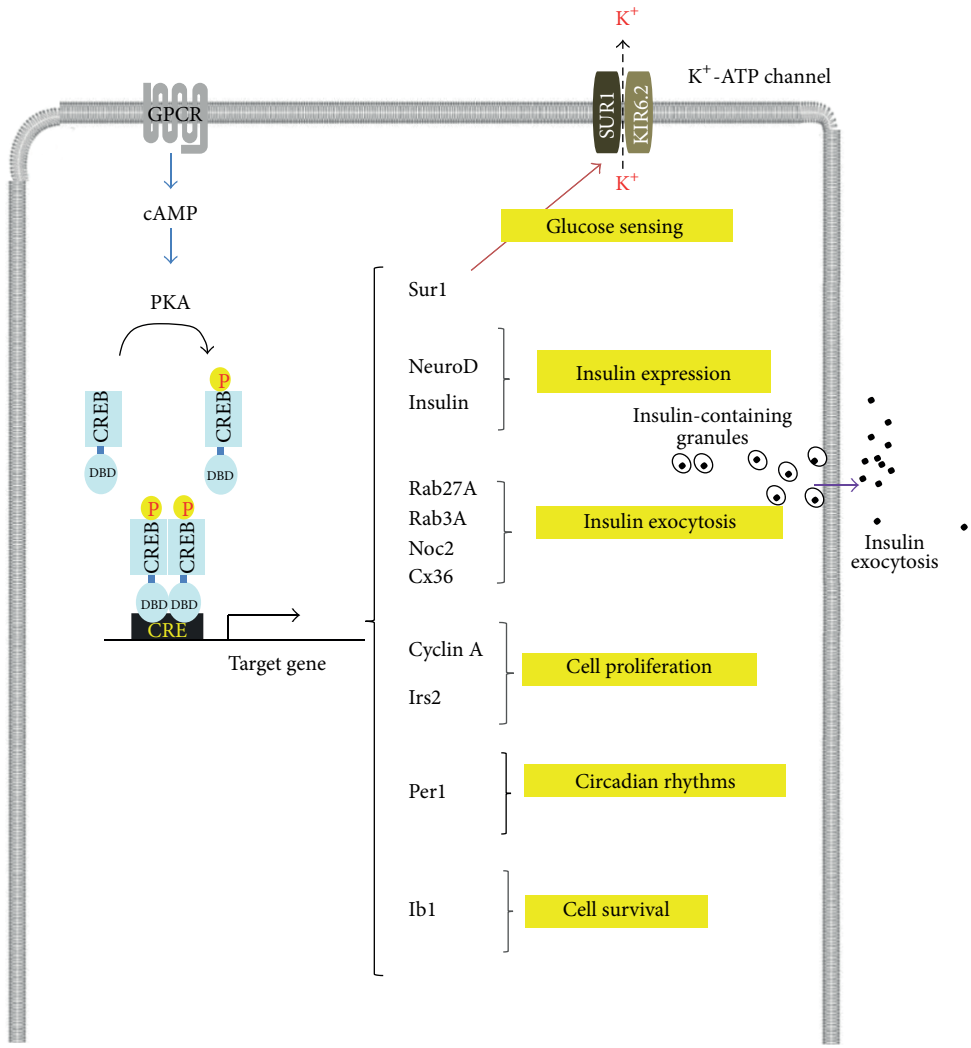

Figure 3: Target genes regulated by CREB and ICER in pancreatic β-cells. Typically phosphorylation of CREB results from the protein kinase A (PKA) activity. PKA activity is stimulated by the G protein coupled receptor-induced increase of cAMP. Some genes regulated by CREB and consequently ICER are listed in the schema. Sur1: sulfonylurea receptor 1; neurogenic differentiation 1: NeuroD; Irs2: insulin receptor 2; Per1: Period 1; Ib1: islet brain 1; Noc2: no C2 domain protein, Cx36: Connexin 36.

and glucose uptake in muscles. Improvement in insulin secretion results from cytoprotective properties of HDL by at least tackling the effects of oxidized LDL. The human modified LDL augments the expression of ICER via oxidative stress [51]. Consequently, elevation of ICER elicited by oxidized LDL cholesterol hampers insulin production and glucose-induced secretion by affecting Rab3A, Rab27A, Slp4, and Noc2. Finally cells cultured with the human oxidized LDL undergo apoptosis because of reduced expression of IB1 and JNK activity.

Transgenic mice that specifically overexpress ICER in β-cells exhibit high blood glucose levels throughout their lifespan and mice died from severe diabetes because of a reduced functional β-cell mass [32]. Chronic hyperglucagonemia usually parallels defective insulin secretion in diabetes. Glucagon acts through stimulation of the cAMP/PKA pathway, resulting in activation of CREB. As the consequence of CREB activity, the expression of ICER is induced, resulting in repression of the insulin gene transcription [52]. Induction of ICER by hyperglucagonemia may represent an additional mechanism contributing to deregulated insulin gene expression and β-cells failure in diabetes.

5. Concluding Remarks and Perspectives

While ICER represses target genes, it inhibits its own promoter as well. This negative feedback loop permits genes expression and ICER as well, returning to basal state. Elevation of ICER observed in islets β-cells exposed to diabetes environmental conditions raises the idea that destruction of ICER is a key for counteracting β-cell failure. This hypothesis is not possible if a systemic approach for silencing ICER in the body is employed. Decline of ICER is detrimental, at least for adipose function and systemic insulin sensitivity. Drastic

reduction in the adipose ICER content, as observed in both obese human and mice, impairs insulin-induced glucose uptake and production of the insulin sensitizer adiponectin [16, 51]. Drop of adiponectin, if protracted in the long term, has adverse effects for systemic insulin sensitivity [53]. A careful examination in the mechanism leading to uncontrolled expression of ICER in β-cells needs therefore to be considered. With this regard, the rise of ICER may result either from increased activators activity or defect of the negative autoregulation. The P2 promoter activity is under the control of CREB. In β-cells exposed to chronic hyperglycemia the CREB level is reduced via proteasomal degradation [54]. A role for CREB in the increased production of ICER seems therefore unlikely. Future studies will be to investigate whether negative regulators are missing or rather some activators are stimulated in diabetes condition to promote sustained expression of ICER. Identification of these mechanisms would pave the way for identification of innovative therapeutic counteracting β-cells dysfunction and death in diabetes.

Conflict of Interests

The authors of this paper have no conflict of interests.

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Review Article

Role of Ink4a/Arf Locus in Beta Cell Mass Expansion under Physiological and Pathological Conditions

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The ARF/INK4A (Cdkn2a) locus includes the linked tumour suppressor genes p16INK4a and p14ARF (p19ARF in mice) that trigger the antiproliferative activities of both RB and p53. With beta cell self-replication being the primary source for new beta cell generation in adult animals, the network by which beta cell replication could be increased to enhance beta cell mass and function is one of the approaches in diabetes research. In this review, we show a general view of the regulation points at transcriptional and posttranslational levels of Cdkn2a locus. We describe the molecular pathways and functions of Cdkn2a in beta cell cycle regulation. Given that aging reveals increased p16Ink4a levels in the pancreas that inhibit the proliferation of beta cells and decrease their ability to respond to injury, we show the state of the art about the role of this locus in beta cell senescence and diabetes development. Additionally, we focus on two approaches in beta cell regeneration strategies that rely on Cdkn2a locus negative regulation: long noncoding RNAs and betatrophin.

1. Introduction

Expansion and proliferation of insulin-secreting beta cells in pancreatic islets is a key highly regulated mechanism for establishing, maintaining, and adapting islet function to meet organism physiological demands. Understanding how the pieces of this mechanism fit together could improve development of islet replacement approaches; given that both type 1 and type 2 diabetes result from reduced beta cell mass and impaired beta cell functions. Islet beta cells expand in neonatal humans, mice, and other species, but this proliferation decays thereafter, which may promote pandemic (type 2) forms of diabetes mellitus [1]. Beta cell mass can be expanded by increasing beta cell replication, enlarging beta cell size, decreasing beta cell death, and promoting beta cell neogenesis. Murine beta cells proliferate slowly after birth, but this process can be accelerated under various conditions, including obesity, pregnancy, and stimulation by different beta cell mitogens, such as glucose, amino acids, insulin, prolactin, placental lactogen, and glucagon-like peptide-1. In addition to differentiation from pre-existing adult beta cell progenitors or transdifferentiation from another cell lineage, beta cell self-replication is considered to be the primary source for new beta cell generation in adult animals [2]. One of the approaches in diabetes research is investigating the function of beta cell cycle activators and inhibitors to elucidate the network by which beta cell replication could be increased to enhance beta cell mass and function.

For the understanding of beta cell replication machinery it is important to focus on the cell cycle phases and checkpoints: the G1-S transition, S phase checkpoint, G2 to M transition and the Mitotic checkpoint (Figure 1). Checkpoint regulation mechanisms act through negative intracellular signals that arrest the cell cycle, rather than through the removal of positive signals that stimulate cell-cycle progression. Negative signals prevent cycle transition at the initiation of proliferation, replication and mitosis until the cellular conditions are the adequate for cell cycle progression. Sequential activation/inactivation of cyclin-dependent kinases (Cdks) is the primary means of cell cycle regulation. Cyclins confer substrate specificity and regulation to Cdk/cyclin complexes. According to that, in early G1 phase Cdk4 and/or Cdk6 are activated by D-type cyclins and initiate phosphorylation

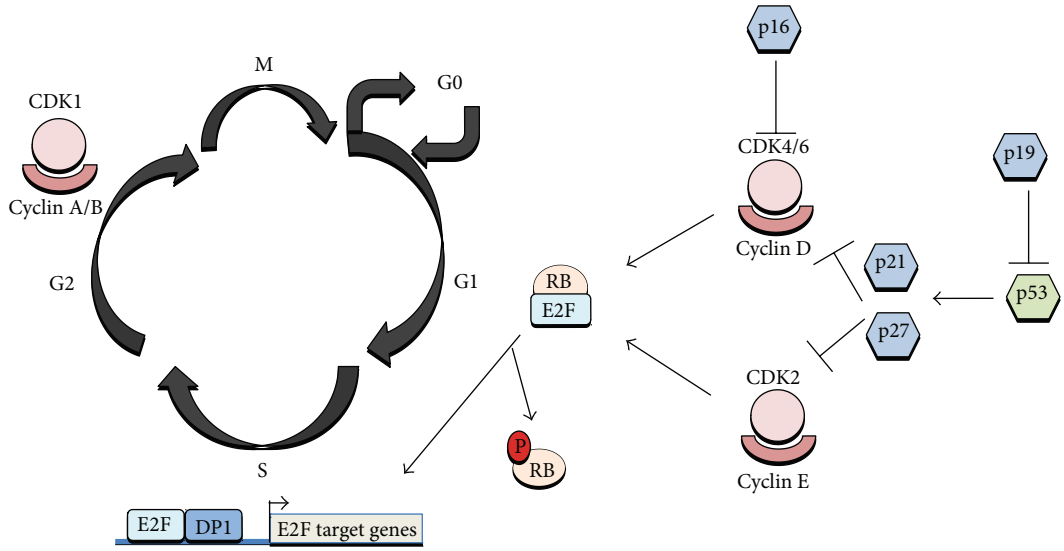

Figure 1: Mammalian cell cycle regulation. $p16^{Ink4b}$ sequesters Cdk4 or Cdk6 inhibiting interactions with type D cyclins and preventing phosphorylation of pRB. Inactivation of CDK4/6 promotes Rb/E2F1 association triggering G1/S transition. Phosphorylation of pRB is essential for passage through the restriction point in G1. The cyclin D1-Cdk4 complex specifically phosphorylates the pRB protein leading to sequential phosphorylation by cyclin E-Cdk2 and release of free E2F. The phosphorylation of pRB, and relief of transcriptional inhibition by pRB induces S-phase entry. p53-dependent regulation by p21 and p27 contributes to checkpoint maintenance at later timepoints. Cdc2-Cyclin A/B binding contribute to phosphorylation of proteins involved in G2/M transition.

of the retinoblastoma protein (Rb) family. This triggers the release of E2F transcription factors and the subsequent activation and transcription of E2F responsive genes (including E- and A-type cyclins) required for cell-cycle progression. In the late G1 phase, Cdk2 binds to cyclin E and completes the phosphorylation of Rb, reinforcing the activation of E2F mediated transcription. These events lead to transition from the G1/S boundary point to S phase initiation.

Through S phase progression Cdk2 binds to cyclin A that contributes to DNA replication. During the G2/M transition, Cdk1/cyclin A complex is required for the initiation of prophase. Finally, Cdk1/cyclin B complexes are actively involved in the completion of mitosis. The negative regulation of Cdk/cyclin complexes relies on two families of Cdk inhibitors; the INK4 family ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, $p19^{INK4d}$) specifically bind to Cdk4 and Cdk6 and prevent D-type cyclin activity and the Cip/Kip family ($p21^{Cip1}$/Waf1/Sdi1, $p27^{Kip1}$, $p57^{Kip2}$) inhibits Cdk2/cyclin E, Cdk2/cyclin A, Cdk1/cyclin A, as well as Cdk1/cyclin B activity (for review see [3]).

The p53 gene product is another key cell cycle check-point regulator at both the G1/S and G2/M and it has been shown to activate transcription of number of cell cycle genes and its essential role is to arrest cells in G1 after genotoxic damage allowing DNA repair prior to DNA replication and cell division. In response to massive DNA damage, p53 triggers the apoptotic cell death pathway [4].

Here we give an overview about the regulation points of ARF/INK4A locus transcription in beta cell, specifically in proliferation processes that give rise to cellular senescence and the development of diabetes. We state the more striking studies that shed a light in the approach of pancreatic beta cell proliferation and regeneration.

2. ARF/INK4A Locus Regulation in Beta Cells

Candidate gene and genome-wide association studies (GWAS) have identified several loci associated with type 2 diabetes and related traits. Specifically, genetic variants at CDKN2A/B locus have been associated with type 2 diabetes in many ethnic populations. A number of publications in the last five years confirm and validate that CDKN2A/B is a locus associated with genetic risk of type 2 diabetes development [5–8]. Among the strongest linked variants is rs10811661, located 125 kb upstream of the CDKN2A and CDKN2B genes (for review, [9]). This polymorphism appears to be associated to type 2 diabetes in almost all the ethnic cohorts studies [10–18] and people carrying the TT genotype of this variant showed impaired insulin release and impaired glucose tolerance [19].

The ARF/INK4A (Cdkn2a) locus spans around 35 kilobases on human chromosome 9p21.25 that includes the linked tumor suppressor genes $p16^{INK4a}$ and $p14^{ARF}$ ($p19^{ARF}$ in mice) that trigger the anti-proliferative activities of both RB and p53. While $p14^{ARF}$ is transcribed from exon 1b and exon 2, $p16^{Ink4A}$ is transcribed from exon 1a localized 20 kb downstream of 1b and exons 2 and 3. As described in more detail below and shown in Figure 2, $p16^{Ink4a}$ binds to CDK4/6 inhibiting its kinase activity thereby preventing Rb phosphorylation (pRb), while $p14^{Arf}$ inhibits the ubiquitin ligase activity of MDM2, thereby stabilizing p53. Rb remains associated with transcription factor E2F1 localizing it to the cytoplasm and thus preventing transcription of E2F1 target genes that, as mentioned above, are crucial for the G1/S transition [20].

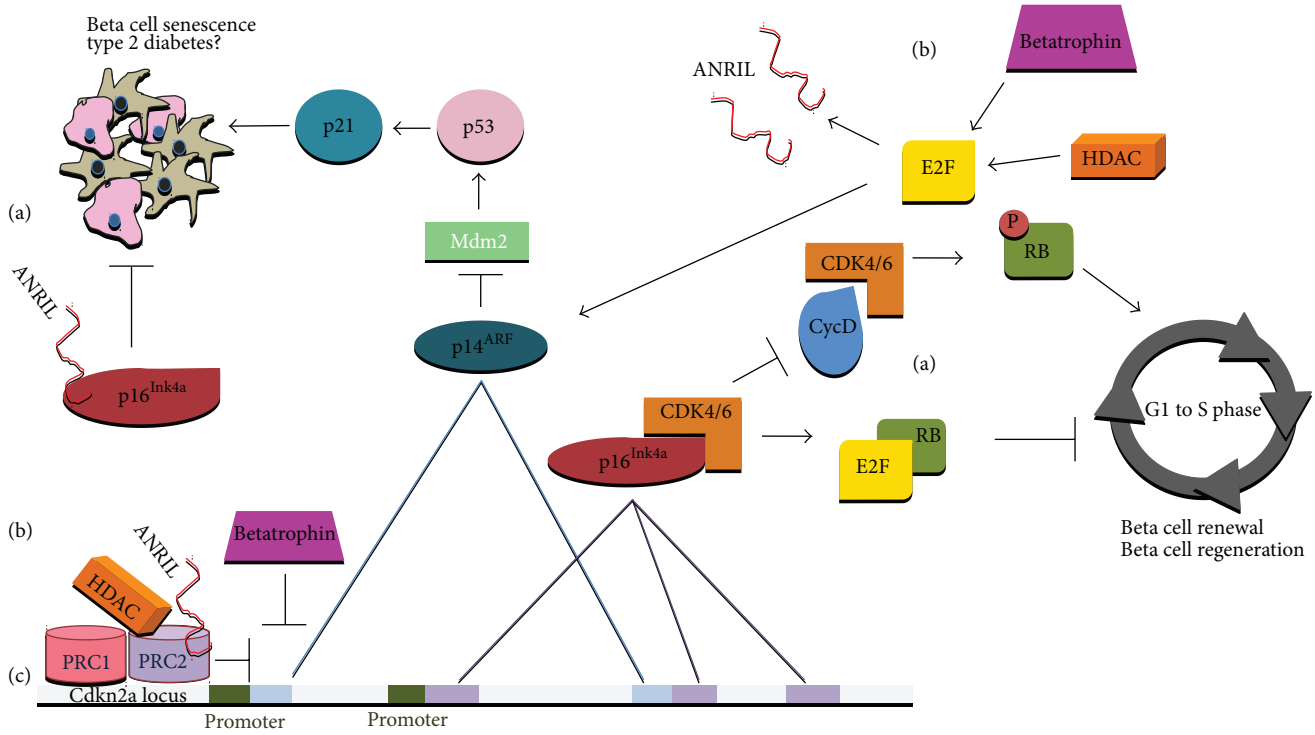

FIGURE 2: (a) Cell cycle regulation by $p16^{Ink4b}/p14^{ARF}$. $p16^{Ink4b}$ inhibits CDK4/6 activity, preventing RB phosphorylation, thus preventing G1/S transition. Inactivation of CDK4/6 promotes Rb/E2F1 association down-regulating transcription of E2F1 target genes, which would trigger G1/S transition. $p14^{ARF}$ inhibits MDM2 activity thereby stabilizing p53 and promoting beta cell senescence. (b) Regulation of beta cell Cdkn2a locus. Binding of PRC1 and PRC2 complex proteins to the p16/p14ARF promoter results in formation of heterochromatin leading to suppression of transcription. HDACs also repress Cdkn2a locus but they conform a regulatory feedback loop: release of E2F1 which up-regulates $p14^{ARF}$ leading to an inhibition of Mdm2 and inducing senescence through p21-p53 pathway. Betatrophin promotes beta cell proliferation by inhibiting Cdkn2a and increasing expression levels of cyclins and E2F1 (through a still unknown mechanism). lncRNA ANRIL, transcribed from the Cdkn2a locus, directly binds to the $p16^{Ink4b}$ transcript and also recruits the PRC complexes to repress the transcription of genes at this locus. ANRIL is induced by E2F1 after DNA damage. (c) Cdkn2a locus showing exons of $p16^{Ink4b}/p14^{ARF}$ genes involved in alternate splicing. Promoter regions are shown in green.

Beta cells express most of the known cell cycle inhibitors, including $p16^{INK4a}$, $p18^{INK4c}$, $p21^{CIP1}$, $p27^{Kip1}$, p53, and Rb. In contrast, and according to the negative regulation model explained in the introduction, there is much less redundancy of cell cycle activators in the beta cell. This model converges in mouse and human models. For example, rodent beta cells express only CDK4 and not CDK6, whereas most other cell types express both of these proteins. Mouse beta cells express all three D cyclins, D1, D2, D3, but the mRNA expression of D2 is significantly higher than both D1 and D3 with only D2 detectable by immunohistochemistry [2]. In order to establish another regulation point of this pathway in beta cells, Fiaschi-Taesch and colleagues [21, 22] have delineated the repertoire of G1/S regulatory proteins present in the adult human islet and have used this information to develop what they call the "human islet G1/S proteome". These studies state that although the G1/S molecules are mainly considered to be nuclear proteins, they are present principally in the cytoplasm, where possibly they would not be able to regulate cell cycle progression. Furthermore, the only nuclear G1/S molecules are the cell cycle inhibitors, pRb, p57, and p21. $p16^{Ink4a}$ remains in the nucleus in only 8.4% of beta cells under basal conditions. Which in turns becomes nuclear in 16.1% under induction of proliferation. Cell cycle activators as cyclins or Cdks, necessary to drive β-cell proliferation are present in the cytoplasm, not in the nuclear compartment.

Regarding the role of E2F in beta cells, it is well known that the increased expression of E2F contributes to the uncontrolled proliferation of cancer cells, but there is increasing evidence for a Cdk4–E2F1–pRB-specific role in metabolism. To that extent, there is the finding that some specific polymorphisms in the Cdk4 gene could contribute to type 2 diabetes-associated obesity [23]. In that sense, studies $E2F1^{-/-}$ mice show impaired postnatal pancreatic growth that triggers a reduction in pancreatic size with the subsequent impaired glucose homeostasis [24]. Moreover, the CDK4-pRB-E2F1 pathway is activated by glucose through the insulin pathway in beta cells, leading to increased Kir6.2 expression that induces insulin secretion [25–27].

3. ARF/INK4A and Beta Cell Senescence

Emerging evidence indicates that proliferation of pancreatic beta cells is an important mechanism not only to maintain

homeostasis in the endocrine pancreas but also for adapting islet function to changes in metabolic demands [9, 28, 29]. The inability of the beta cells to expand and compensate for the changing insulin demand can contribute to the pathogenesis of diabetes. Several studies suggest that beta cell proliferation declines with age [30, 31] and this age-dependent decline in the beta cell proliferation could curtail the ability of the endocrine pancreas to respond to metabolic changes. Furthermore, the cell-intrinsic genetic and epigenetic mechanisms regulating the age-dependent decline of beta cell proliferation [32].

On their review, Gunasekaran and cols compile contradictory studies on the age-related effects on the beta cell. While some of them found that insulin sensitivity decreases with age, others shown that plasma glucose clearance was found to be dependent on the waist-to-hip ratio and not age, with the exception of older people with pre-existing impaired glucose tolerance or type 2 diabetes. Even if these studies come into contradiction, there is evidence that with age, beta cells show decreased expression of cell cycle activators with simultaneous increases in expression of cell cycle inhibitors. Controversial findings and opinions regarding the balance of cell cycle inhibitors and activators have been found. Gunasekaran's compendium describes several studies showing that loss of a single cell cycle inhibitor does not accelerate beta cell cycle progression, whereas loss of multiple inhibitors enhances beta cell proliferation [2]. In the opposite sense, other groups describe that $p16^{Ink4a}$ and $p19^{Arf}$ expression (mRNA) was increased significantly with aging in pancreatic islets, but not other Cdk inhibitors examined, including p15, p18, p21, p27. This up regulation has been linked to reduction in the proliferative capacity of aged beta cells [32, 33].

Aging is associated with replicative senescence and $p16^{Ink4a}$ levels increase with aging in most mammalian tissues. The levels of $p16^{Ink4a}$ in an individual can be predicted by stochastic model that takes into consideration the subjects' age. According to this model, $p16^{Ink4a}$ levels exponentially increase with age in a $p16^{Ink4a}$-dependent manner and reach a plateau.

Increased $p16^{Ink4a}$ levels in the pancreas during aging (independent of telomere shortening) inhibit the proliferation of beta cells and decrease their ability to respond to injury. While the beta cells of the $p16^{Ink4a}$ knockout mice were able to proliferate in response to injury, beta cells with ectopic expression of $p16^{Ink4a}$ showed reduced proliferative response confirming the association between $p16^{Ink4a}$ and beta cell senescence [20]. In addition, overexpression of $p16^{Ink4a}$ in transgenic mice caused a reduction of islet proliferation in younger more than in older animals.

Expression of $p16^{Ink4a}$ and $p14^{ARF}$ are regulated by promoter hypermethylation through proteins of the PRC1 and PRC2 complexes of the Polycomb group (PcG) of transcriptional repressor proteins. MLL1 and PcG directly control the Ink4a/Arf locus through chromatin epigenetic modifications and the loss of these repressive epigenetic marks leads to a shift of the replication timing of the locus, both in senescent and Polycomb mutant cells [34].

Bmi-1 is a transcription factor, member of the PcG repression complex 1 (PRC1) that inhibits senescence by inhibiting transcription of $p16^{Ink4a}$. Thus Bmi-1 behaves as an oncogene and is a marker of tumor stem cells. Ezh2 (Enhancer of zeste homolog 2) belongs to the PRC2 complex, it is a histone methyltransferase which represses Ink4a/Arf in islet beta cells, with activity specific for histone H3 K27 [35, 36]. The ability of Bmi-1 to decrease transcription from the Ink4a locus depends on the presence of Ezh2 and other components of the PRC2 complex.

Kotake et al. [37] showed that the removal of pRb from cells resulted in the loss of histone H3 K27 trimethylation leading to the loss of Bmi-1 recruitment to the Ink4a/Arf locus. Moreover, pRb is also shown to be necessary for Bmi-1 function in the transcription repression of Ink4a/Arf. There is a feedback loop between $p16^{Ink4a}$ and Rb: phosphorylation which results in increased $p16^{Ink4a}$ expression and inhibition of CDK4/6 [20]. Other regulatory feedback loop of the locus relays on histone deacetylases (HDAC), p53 is required for both HDAC and PcG to repress $p14^{Arf}$ expression [38], at the same time HDAC1 is involved in the release of E2F1 which in turn could up-regulate $p14^{Arf}$, leading to an inhibition of Mdm2 activity, the subsequent activation of p53 and induction of senescence through p21 [20, 39]. Previously described by Wang and colleagues [40], HBP1 induces premature senescence through upregulating $p16^{Ink4A}$ expression in primary cells by targeting the $p16^{Ink4A}$ promoter, by interacting and recruiting p300/CBP, whereas HDAC4 represses HBP1-induced $p16^{Ink4a}$ expression, thus represses HBP1-induced premature senescence.

4. New Insights into INK4a/ARF Regulation of Beta Cell Proliferation

The factors explained below are well known to have a specific role in regulation of beta cell proliferation under physiological and pathological conditions. However, the molecular pathways in which they are involved are not fully studied, but they could be promising targets for expanding functional pancreatic islets in diabetes.

5. lncRNAs

Long non-coding RNAs (lncRNAs) are a new class of regulatory RNAs that are defined as transcribed RNA molecules ranging in length from 200 to 100,000 nucleotides and lacking protein-coding capacity. Islet lncRNAs show a marked cell-type specific expression pattern, Morán and colleagues [41] have integrated transcriptional and chromatin maps to systematically annotate lncRNA genes in human pancreatic islet cells and they state that some lncRNAs are dysregulated in type 2 diabetes or map to susceptibility loci. Furthermore, orthologous transcripts in mice are dynamically regulated in a similar manner as human islet lncRNAs. In the case of Cdkn2a, a long non-coding RNA, ANRIL (antisense non-coding RNA), also transcribed from the locus, is involved in the epigenetic regulation of the Cdkn2a locus by direct

binding to the $p16^{Ink4b}$ transcript and recruiting the PRC complexes to repress the transcription of genes at this locus. ANRIL is targeted by PRC2 to the Ink4a/Arf/Ink4b locus [42]. Another regulation point regarding ANRIL and cell cycle inhibition is that ANRIL is induced by E2F1 transcription factor after DNA damage, and thus the elevated ANRIL levels suppress the expression of $p14^{Arf}$ and $p16^{Ink4a}$ at the late-stage of DNA damage response (DDR), forming a negative feedback loop to the DDR [43]. Recent studies showed that single nucleotide polymorphisms mapped in the ANRIL as well as in Cdkn2a locus sequences are linked to several pathologic conditions, including type 2 diabetes [39].

6. Betatrophin

Yi et al. [44] have described a peptide whose overexpression in mouse liver produces a secreted protein that significantly and specifically promotes pancreatic beta cell proliferation and beta cell mass expansion and, consequently, improves glucose tolerance. Hence, this peptide has been called betatrophin. Expression levels of cyclins (cyclins A1, A2, B1, B2, D1, D2, and F), CDKs (CDK1 and CDK2), and E2Fs (E2F1 and E2F2) increase, whereas cell cycle inhibitors (Cdkn1a and Cdkn2a) decrease in islets of betatrophin-injected mice compared to control-injected mice. The mechanism of action for betatrophin still remains unknown, if it acts directly or not on the beta cells for controlling their proliferation. Betatrophin is not a novel protein, it is also known as ANGPTL8, TD26, RIFL and Lipasin. Previously to the study of Yi and colleagues, it has been shown that betatrophin is expressed at the highest levels in liver and adipose tissue, and is up-regulated by feeding and suppressed by fasting [45, 46]. To date the betatrophin receptor has not been identified, and maybe other cofactors are acting in the specificity of the betatrophin effect on beta cell mass. However, promising opportunities are open with regard to betatrophin and beta cell mass regeneration.

7. Conclusions

Type 1 and 2 diabetes can be reversed by replacement of beta cell mass, as demonstrated by pancreas and islet transplantation [47]. However, it has limited applicability, given the shortage of organ donors and the need for chronic immunosuppression. Regeneration of beta cell mass is one promising approach to replace the deficit in beta cell mass in diabetic patients [32]. The results stated in this review support that modulation of the Ink4a/Arf locus plays a critical role in regulating pancreatic beta cell proliferation during aging and regeneration. A strategy of beta cell proliferation improvement based in Ink4a/Arf genes inhibition could help to develop new regeneration approaches. One of the main concerns about inhibiting the action of these genes is that they are anti-oncogenes, and could trigger tumour development [48]. To date, few studies show specific inhibitors of p16 and/or p19 genes. Recently, the direct derepression of p21 and $p16^{Ink4a}$ caused by loss of AP4 gene (a c-Myc transcription factor) in fibroblasts has been shown to be sufficient to mediate cellular senescence [49]. In beta cells, a recent and promising study shows that a bioavailable HNF4α (a nuclear receptor transcription factor) antagonist induced β-cell replication in rabbits and mice. Moreover, this compound promotes alpha, beta and delta cell replication in beta cell ablated mice, and repressed the expression of multiple cyclin-dependent kinase inhibitors, including $p16^{Ink4a}$ [50]. Understanding the regulation of Ink4a/Arf locus could reveal the molecular basis of reduced beta cell proliferation with aging and also be extremely useful in devising strategies for beta cell regeneration.

Conflict of Interests

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

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