Newer perspective on the coupling between glucose-mediated signaling and β -cell functionality

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Abstract. Insulin secretion by the pancreatic β -cells is elicited in response to elevated extracellular glucose concentration. In addition to triggering insulin secretion, glucose-induced signal regulates β -cell proliferation and survival. However, the molecular mechanism underlying the effects of glucose on the β -cell functionality still remains unclear. Glucokinase, a hexokinase isozyme that catalyzes the phosphorylation of glucose, acts as the glucose sensor in the β -cells. To investigate the mechanisms of glucose signaling in the regulation of β -cell functions, we analyzed the role of glucokinase in insulin secretion, β -cell proliferation and β -cell apoptosis, using β -cell-specific glucokinase-haploinsufficient ($Gck^{+/-}$) mice and allosteric glucokinase activators (GKAs). Glucokinase-mediated glucose metabolism (1) suppresses endoplasmic reticulum (ER) stress-induced β -cell apoptosis *via* inducing insulin receptor substrate-2 (IRS-2) expression and expression of ER stress-related molecules, (2) promotes adaptive β -cell proliferation through activation of the Forkhead Box M1 (FoxM1)/polo-like kinase-1 (PLK1)/centromere protein-A (CENP-A) pathway, (3) induces islet inflammation by promoting interaction of islet-derived S100 calcium-binding protein A8 (S100A8) with macrophages, (4) induces the expression of Fibulin-5 (Fbln5), an extracellular matrix protein to regulate β -cell functions, and (5) activates other unknown pathways. Glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors have been found to possibly compensate for dysregulation of glucose metabolism in the β -cells. This review provides an update and overview of the recent advances in the study of β -cell pathophysiology and some therapeutic possibilities focusing on glucose-/glucokinase-mediated signaling.

Key words: Glucose, Glucokinase, Pancreatic β -cell, Diabetes

Introduction

Pancreatic β -cells play a pivotal role in maintaining the blood glucose levels within a narrow physiological range through regulating insulin secretion. Reduction in β -cell functions and/or mass is observed in both individuals with type 1 and type 2 diabetes [1, 2]. Thus, enhancing β -cell functionality has the potential to overcome significant challenges in the cure and care of diabetes.

The glycolytic pathway is initiated by a hexokinase that converts glucose to glucose-6-phosphate in the cells. Glucokinase, the rate-limiting enzyme of the glucose oxidation reaction, which acts as a glucose sensor in the β -cells, belongs to the hexokinase family and is predominantly expressed in the liver, β -cells, and neuroendocrine cells [3, 4]. It plays a crucial role in the maintenance of

glycemic control through regulation of insulin secretion and conversion of glucose into glycogen in the liver. Mutations of the human glucokinase gene are responsible for maturity-onset diabetes of the young (MODY)-2, a specific diabetes phenotype characterized by mild hyperglycemia [5, 6].

Glucose metabolism initiated by glucokinase in the β -cells was an obligatory step for a compensatory increase of the β -cell mass in obese mice induced by high-fat diet [7] and an increased expression of insulin receptor substrate-2 (IRS-2) through the CREB, calcineurin/nuclear factor of activated T cell (NFAT), or ChRBP pathways [8-10]. Activation of glucokinase induces upregulation of IRS-2, which leads to activation of β -cell proliferation on one hand [7, 11], apoptosis on the other hand: the latter is in part due to altered interaction with mitochondria, Fas receptor expression, endoplasmic reticulum (ER) stress, oxidative stress, and/or glycogen accumulation [12-16]. Hyperactivation of glucokinase is associated with both enhanced β -cell replication and apoptosis in normal and diabetic individuals [17, 18]. Diet-induced metabolic changes have previously been investigated in β -cell-specific glucokinase-

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Fig. 1 Previously known pathways of glucose metabolism in the β -cells.

Glucose metabolism is responsible for glucose-induced insulin secretion *via* glucokinase-mediated glycolysis, TCA cycle, K_{ATP} channel, and Ca²⁺ influx. Insulin signaling is activated by glucose signaling *via* IRS-2 expression or insulin receptor activation, which also enhances β -cell proliferation through CREB, calcineurin, and ChREBP. Chronic glucokinase activation promotes apoptosis through multiple pathways, including oxidative stress and ER stress, Fas receptor expression, or glycogen storage.

haploinsufficient (*Gck*^{+/-}) diabetic mice [19, 20]. *Gck*^{+/-} mice were given a diet containing a combination of sucrose and linoleic acid (SL), which resulted in a reduction of the β -cell mass due to increased β -cell apoptosis. SL-fed *Gck*^{+/-} mice showed increased expression levels of C/EBP-homologous protein (CHOP) and SREBP-1c in the pancreatic islets. Therefore, haploinsufficiency of glucokinase promoted β -cell apoptosis *via* dietary fatty acid-induced ER stress. These findings prompted us to consider the possible existence of previously unknown mechanisms in the regulation of β -cell function and mass by glucose-derived signal (Fig. 1).

In this review, we shall focus on the changes in the glucokinase activity and the consequences; remote metabolic alterations induced by glucose are out of the scope of this communication.

Effects of GLP-1 Receptor Signaling in Glucokinase-deficient β -cells

Glucagon-like peptide-1 (GLP-1) binds with its receptor facilitating glucose-dependent stimulation of insulin secretion from the pancreatic β -cells [21]. However, the role of glucokinase in the regulation of GLP-1 receptor-mediated signaling in the β -cells still remains unknown. The effects of liraglutide, a GLP-1 receptor agonist, on the β -cell functions in β -cell-specific *Gck*^{+/-} mice and β -cell-specific glucokinase-deficient (*Gck*^{-/-}) newborn mice were investigated in a previous study [22]. Liraglu-

tide reduced the blood glucose levels in the *Gck*^{+/-} mice without changing insulin secretion. GLP-1-induced increase of insulin secretion is known to be mediated by the autonomic nervous system [23]. Thus, the mice were treated with chlorisondamine (CS), a nicotine acetylcholine receptor antagonist and a neuronal and ganglionic blocker, or methylatropine (Atr), a peripheral muscarinic receptor blocker. Although the serum insulin levels decreased following administration of CS and Atr after glucose loading, liraglutide significantly reduced the blood glucose levels, irrespective of the administration of CS or Atr, in both wild-type and *Gck*^{+/-} mice, suggesting that the glucose-lowering effect of liraglutide is mediated, at least in part, by extrapancreatic effects (*e.g.*, gastrointestinal motility). Liraglutide also improved hyperglycemia, fatty liver, and β -cell loss in *Gck*^{-/-} neonates [22]. Thus, β -cell glucokinase may not be a prerequisite for GLP-1 to lower glucose, to alleviate hepatic steatosis, and to prevent β -cell death, even though it is required for GLP-1-induced increase of insulin secretion from the pancreatic β -cells. In accordance with these findings, the favorable effects of incretins on the β -cell survival were present in the β -cells of *Gck*^{-/-} mice [20, 22]. Extrapancreatic effects of dipeptidyl peptidase 4 (DPP-4) inhibitors have also been demonstrated [19, 24-26]. Because DPP-4 *per se* is expressed in the β -cells and plays a role in β -cell functions, DPP-4-mediated signaling is potentially related to glucose metabolism in the β -cells [27].

Glucokinase Activation and ER Stress–Induced Apoptosis of the Pancreatic β -cells

ER stress could predispose to the development of diabetes or metabolic syndrome through inducing β -cell apoptosis, hypothalamic dysregulation, and insulin resistance [28–30]. *Gck*^{+/-} mice fed a diet rich in linoleic acid and sucrose showed increased β -cell ER stress and apoptosis as compared to the wild-type mice fed the same diet [20]. Therefore, we investigated the role of glucokinase in the regulation of ER stress-induced apoptosis of the β -cells [31]. Small-molecule glucokinase activators (GKAs) activate glucokinase by binding to the allosteric site of the glucokinase enzyme [32, 33], and facilitate β -cell proliferation by enhancing glucose metabolism in the β -cells [17, 34]. GKAs were shown to ameliorate dysglycemia and attenuate β -cell apoptosis in Akita mice carrying a heterozygous Cys96Tyr mutation in the Insulin 2 gene and increased β -cell ER stress and cellular apoptosis [35, 36]. Then, the role of IRS-2, which is known to be upregulated by glucokinase activation, in ER stress-induced β -cell apoptosis was investigated [31]. Glucokinase activation by a GKA or high blood glucose increased the expression of IRS-2 under ER stress, both *in vivo* and *in vitro*. As compared to Akita mice, a significant increase of β -cell apoptosis was observed in both β -cell-specific *Gck*^{+/-} Akita mice and IRS-2-knockout Akita mice, whereas a significant reduction of β -cell apoptosis in the β -cell-specific IRS-2-overexpressing Akita mice. Glucokinase activation by a GKA modulated the expression of ER stress-related genes, such as CHOP, Stc2, Ero-1 β , Sdf211, and Edem-2 (reported in [36–40]), in an IRS-2-independent manner in the β -cells, which led to ERK1-/ERK2-dependent reduction in the expression of Bcl2-associated X protein (Bax). Thus, glucokinase activation was found to protect against β -cell apoptosis under ER stress through upregulation of IRS-2 and also IRS-2-independent downregulation of ER stress-related genes [31].

Insulin Receptor- and IGF-1 Receptor-Independent β -cell Proliferation Induced by Glucose-mediated Signaling

Glucose infusion into mice *in vivo* and direct glucose stimulation of both mouse and human islets *in vitro* induced β -cell replication, in spite of the fact that β -cell proliferative activity in the human islets is quite limited [41, 42]. This glucose-induced β -cell proliferation is reportedly mediated by IRS-2, mammalian target of rapamycin (mTOR), and cyclin D2, but not the insulin receptor (IR) [43]. The effect of OSI-906 (linsitinib), a dual inhibitor of the IGF-1 receptor (IGF-1R) and IR

[44], was examined on the β -cell proliferation *in vivo* [44]. Oral administration of OSI-906 caused marked hyperglycemia and hyperinsulinemia in the mice within a day, and administration of OSI-906 for a week resulted in a significant increase of the β -cell mass and β -cell proliferative activity, without any alterations of insulin signaling in the β -cells. Hence, blockade of IGF-1R and IR promoted the hyperglycemia-induced adaptive β -cell proliferation leading to hyperinsulinemia. Interestingly, a 7-day withdrawal of OSI-906 reduced the severity of the hyperglycemia and of the observed increment in the β -cell proliferative activity [45]. Thus, adaptive β -cell proliferation induced by glucose-mediated signaling is reversible. Glucose signaling has also been shown to cause nuclear export of FoxO1 in IR-deficient β -cells [46].

Glucose-induced Adaptive β -cell Proliferation via the FoxM1/PLK1/CENP-A Pathway

β -cell mass is increased by adaptive β -cell proliferation, upon induction of insulin insensitivity, to maintain the blood glucose levels within the physiological range in rodents. The expressions of genes encoding molecules involved in insulin receptor-mediated signaling were downregulated in human islets isolated from donors with type 2 diabetes, as compared to the islets isolated from non-diabetic control subjects [47–49]. Therefore, we investigated islets isolated from β -cell-specific insulin receptor-knockout (β IRKO) mice as a model of human type 2 diabetes [50]. The β IRKO mice showed impaired compensatory β -cell proliferation in response to DIO or hepatic insulin resistance resulting in a reduced functional β -cell mass [51]. It was found that the β -cells in the islets of the β IRKO mice showed M-phase arrest and significant downregulation of M-phase-related gene expressions. Among these, two M-phase related proteins, namely, centromere protein-A (CENP-A) and polo-like kinase-1 (PLK1), are known to be regulated by the transcription factor FoxM1, downstream of insulin signaling. Hence, the FoxM1/PLK1/CENP-A pathway plays a crucial role in adaptive β -cell replication in response to aging, DIO, pregnancy, or acute insulin resistance induced by S961, an insulin receptor-antagonist, via the insulin receptor-mediated signaling pathway [52]. The significance of CENP-A in glucose-induced β -cell proliferation was also examined in the report. CENP-A-deficient β -cells showed reduced β -cell replication in response to GKA stimulation [52]. Consequently, the FoxM1/PLK1/CENP-A pathway is a pivotal regulator of adaptive β -cell proliferation induced by glucokinase-initiated glucose signaling.

Glucose-mediated Signaling in the β -cells Induces Islet Inflammation *via* S100A8

In individuals with type 2 diabetes, islet inflammation due to macrophage infiltration is responsible for β -cell apoptosis and decline of the β -cell mass [53]. We demonstrated that glucose-derived signaling in the β -cells robustly upregulated the expression of S100 calcium-binding protein A8 (S100A8) [54], a member of damage-associated molecular pattern molecules (DAMPs), which is known to be involved in the pathogenesis of various diseases, including cancer, inflammatory, skin, and metabolic disorders [55–58]. Exposure to a combination of high glucose, palmitate, and macrophages has been shown to synergistically upregulate the expression of S100A8 in both mouse and human islets, independent of Toll-like receptor 4 (TLR4) [54]. In turn, the β -cell-derived S100A8 potentiates macrophage migration and inflammatory cytokine production through TLR4 activation on macrophages, resulting in islet inflammation and β -cell apoptosis [54]. These findings indicate that S100A8 is a key mediator of islet inflammation under the diabetic hyperglycemia, dyslipidemia, and inflammation with macrophages, and could be a therapeutic target to protect β -cells against gluco- and lipotoxicity.

Fibulin-5 Is a Target Molecule of Glucokinase-mediated Signaling to Regulate β -cell Function

The results of gene expression microarray analysis in GKA-stimulated islets revealed that glucokinase activation significantly augmented the expression of Fibulin-5 (Fbln5) [59], a secreted matricellular protein, which is known to play a crucial role in the assembly of elastic fibers [60]. All of a glucokinase inhibitor, K_{ATP} channel opener, Ca^{2+} channel blocker, and calcineurin inhibitor blunted the upregulation of Fbln-5 induced by glucose-mediated signaling in the β -cells [59]. Conversely, harmine, a dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) 1A inhibitor, enhanced the induction of Fbln-5 through glucose-mediated signaling [59], suggesting that the expression of Fbln5 is mediated by the calcineurin/NFAT pathway. Overexpression of Fbln5 has been reported to be responsible for elevated glucose-stimulated insulin secretion in rat INS-1 cells [59]. Currently, analysis on β -cell-specific Fbln5-knockout mice is underway to further investigate the role of Fbln5 in the β -cells. In conclusion, glucose mediated signaling in the β -cells may also affect the surrounding elastic fibers to control β -cell functions. This concept warrants further exploration to uncover the molecular aspects of novel glucose signaling in and around the β -cells.

Other Potential Pathways Downstream of Glucose Metabolism in the β -cells

Other previously unrecognized targets of glucokinase in the β -cells have been identified, including neuronal pentraxin 2 (Nptx2), tweety homolog 1 (Ttyh1), secretory leukocyte peptidase inhibitor (Slpi), semaphorin 3C (Sema3c), doublecortin-like kinase 1 (Dlck1), and tachykinin 1 (Tac1) [31]. Therefore, we believe whole picture of glucose-mediated signaling in the β -cells remains to be elucidated. There above described ones could be candidate genes to be evaluated for their role in glucose metabolism in the β -cells.

As well known, glucose-mediated signaling is closely linked to insulin signaling in the β -cells, in the context of β -cell proliferation and survival [7]. In insulin receptor-deficient β -cells, GLP-1 receptor signaling improved the impaired insulin signaling and upregulated cyclin gene expression, resulting in β -cell proliferation [25]. It has also been shown that glucose-mediated signaling affects incretin receptor expression through phosphorylation of the AMP-activated protein kinase (AMPK) [61]. This result suggests that glucokinase activation by GKAs and AMPK phosphorylation by metformin coordinately regulate the responsiveness to incretins, DPP-4 inhibitors, and GLP-1 receptor antagonists.

Glucokinase has been known to be localized in the mitochondria, regulates apoptosis in the β -cells [12, 62], and is regulated by the glucokinase regulatory protein (GKRP), a competitive inhibitor of glucokinase for glucose binding in the liver [63]. Interestingly, we found glucokinase translocation from the nucleus to the cytosol upon glucose-dependent dissociation from GKRP in hepatocytes. However, the nuclear localization of glucokinase in β -cells has been controversial. The possibility that SUMOylation of glucokinase modulates its nuclear import in the β -cells has been demonstrated [64]. Thus, intracellular localization of glucokinase might provide us with novel insights into the pathways and processes of glucose metabolism in the β -cells.

Conclusions and Future Perspectives

Glucotoxicity is a type of cell damage caused by chronic activation of the β -cells with high blood glucose levels. With glucotoxicity, the expression level of glucokinase is reduced in the β -cells. While glucokinase activation protects β -cells from ER stress, there may be two-sided effects of glucose metabolism to determine the cell fate by glucose metabolism.

Human and mouse islets differ in terms of their structure, composition, functions, and gene expression profile [65] and therefore, glucose signaling possibly has differ-

ent responses in human and mouse islets. Accordingly, an understanding the mechanism(s) modulating human β -cell functions and mass is essential when designing therapeutic strategies against diabetes in humans [66]. In this regard, heterozygous activating glucokinase mutations in human β -cells exhibits unique role of glucokinase for increased β -cell mass because patients with this mutation, familial hyperinsulinemic hypoglycemia, show not only hypersecretion of insulin, but also increased β -cell mass, enhanced β -cell proliferation, and elevated β -cell apoptosis [18, 67].

In vivo regulation of islet cell function and mass *via* organ crosstalk should also be considered in implementing a strategy for restoration of the functional β -cell mass [68]. Hyperglycemia causes hyperinsulinemia, hyperlipidemia, an increase in the serum levels of advanced glycation end products (AGEs), or other metabolic alterations. In addition to glucose, and insulin affects metabo-

lism in the liver, adipose tissue, muscle, brain, and β -cells. Mice with systemic insulin signaling blockade with S961 or OSI-906, which show severe hyperglycemia and marked hyperinsulinemia, are models of interest to investigate β -cell proliferation in terms of glucose metabolism and inter-organ crosstalk with β -cells [44, 69]. Recent progress has indicated that some different subtypes of β -cells are present and each subtype shows functional heterogeneity in terms of the glucose sensitivity [70-72]. Hence, the signaling pathways for glucose metabolism may differ between the subtype of β -cells.

The findings presented here indicate that glucose-mediated signaling is clearly important for the maintenance of healthy β -cell functions and mass, *via* newly identified mechanisms (Fig. 2). This classical, but unresolved glucose signaling in the β -cells provides a strong rationale for further investigations in this area of research in order to develop effective treatments for diabetes.

Fig. 2 Novel pathways of glucose metabolism in the β -cells.

Glucose signaling ameliorates ER stress-induced β -cell apoptosis through combination of ERK-mediated reduction in CHOP, modification of ER stress-related gene expression, or IRS-2 activation. Glucose metabolism enhances adaptive β -cell proliferation through the FoxM1/PLK1/CENP-A pathway. Glucokinase-mediated glucose signaling also regulates matricellular protein Fbln-5 expression *via* NFAT, and inflammatory protein S100A8 expression *via* Ca^{2+} . Both of these molecules, involved in extracellular matrix regulation of islet function and islet inflammation. Glucokinase could potentially translocate to the nuclei to regulate the β -cell functions.

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Disclosure

None of the authors have any potential conflicts of interest to disclose in relation to this research.

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