Glucose- or insulin resistance-mediated β-cell replication: PKCζ integrates the proliferative signaling

The concern over global epidemics of diabetes and obesity has risen. Obesity is a conspicuous risk factor for the development of type 2 diabetes, and with the global obesity rates rising, diabetes can be considered as a global public health problem. The aging of society, lifestyle changes and epigenetic modifications have all caused a rapid increase in diabetes prevalence, especially in Asian countries. One explanation for the large impact of lifestyle changes on diabetes prevalence is the change in dietary habits. Diets containing high amounts of fat can easily provoke insulin resistance in obesity-prone Asian individuals. When adaptive β-cell proliferation and β-cell mass expansion are insufficient to produce sufficient insulin to overcome diet-induced insulin resistance, type 2 diabetes is manifested. A progressive decline in the insulin-producing β-cell volume is an important feature of the type 2 diabetes pathophysiology, prompting further investigation of the mechanisms underlying the regulation of the β-cell mass. Some studies have already shown that adaptive β-cell replication is observed after short-term high-fat diet (HFD) feeding, before insulin resistance and obesity develop. This represents the reasonable physiological response against diabetes onset. A human autopsy study has suggested that obese individuals without diabetes have higher β-cell masses as compared with lean non-diabetic individuals or obese individuals with diabetes. Thus, mice with HFD-induced obesity might serve as a useful physiological model of β-cell proliferation and mass expansion to study the pathophysiology of human type 2 diabetes.

Glucokinase (Gck) acts as a glucose sensor in pancreatic β-cells, and plays a central role in glucose-stimulated insulin secretion. β-cell-specific Gck-heterozygous-knockout mice showed decreased β-cell replication after HFD feeding, resulting in insufficient adaptative increment of the β-cell mass[1]. Glucokinase activators have been developed as antidiabetes drugs, targeting insulin release from β-cells and glucose utilization in the liver[2]. Glucokinase activation initiates β-cell proliferation and blocks β-cell apoptosis, at least in part through the insulin receptor substrate-2 (IRS-2)-mediated insulin signaling pathway[2]. Indeed, β-cell-specific IRS-2-heterozygous-knockout mice failed to show any increase of the β-cell mass under the condition of HFD-induced insulin resistance[1]. Conversely, β-cell-specific IRS-2 transgene overexpression rescued HFD-fed Gck-haploinsufficient mice from hyperglycemia by increasing the β-cell mass[1]. Thus, this integration of glucose signaling and insulin signaling enables the β-cells to show adequate proliferation under the condition of diet-induced obesity. However, the mechanisms underlying this adaptive β-cell replication are not yet fully understood.

In a recent report, Lakshmipathi et al.[3] elucidated the role of protein kinase Cζ (PKCζ), an atypical PKC, in rodent β-cell proliferation induced by glucose stimulation or HFD feeding. They carried out in vitro culture experiments using rodent and human islets and the INS-1 rat β-cell line, and in vivo glucose infusion or HFD-fed mouse models. They were the first to show that glucose stimulation induced PKCζ phosphorylation (activation) and β-cell replication in mouse islets, INS-1 cells and human islets. Both of these effects were blunted by treatment with a phosphoinositide 3-kinase (PI3K) inhibitor, suggesting that glucose-induced PI3K activation is a prerequisite for PKCζ phosphorylation in β-cells and β-cell proliferation. Notably, stimulation with exogenous insulin also upregulated PKCζ phosphorylation in the β-cells. Overexpression of kinase-dead (KD)-PKCζ (K281W) repressed the glucose- and glucokinase activator-induced β-cell replication in the INS-1 cells, mouse islets, and human islets. They also generated a transgenic mouse that expressed KD-PKCζ in the β-cells under the rat insulin promoter. The adult transgenic mice showed no significant changes in the blood glucose levels, plasma insulin levels, glucose tolerance, insulin sensitivity or β-cell mass as compared with wild-type mice under basal conditions. However, glucose infusion was associated with a significant decrease of the β-cell proliferative response in the KD-PKCζ transgenic mice, even though similar blood glucose and serum insulin levels were observed during the glucose infusion. Thus, PKCζ is crucial for glucose-induced adaptive β-cell replication, but not for maintenance of the β-cell mass under the physiological state. Similar results were also seen in HFD-fed mice: overexpression of KD-PKCζ in β-cells reduced β-cell proliferation, but had no effects on the blood glucose or serum insulin levels. The authors also created tamoxifen-inducible β-cell-specific PKCζ-knockout mice. Interestingly, PKCζ expression in β-cells is not essential to

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maintain β-cell replication in normal-chow-fed mice. Meanwhile, PKCζ-knockout mice showed reduced β-cell replication in response to HFD feeding, without altering the blood glucose or serum insulin levels. These results suggest that PKCζ plays a crucial role in glucose- and HFD-induced β-cell proliferation.

How does PKCζ transduce the signals from glucose or HFD feeding to the β-cells to induce their replication? Cyclin D2 has been shown to be essential for adaptive β-cell proliferation induced by glucose or HFD in rodents[1]. Lakshmipathi et al.[3] showed that overexpression of KD-PKCζ blunted glucose-induced cyclin D2 expression in INS-1 cells and mouse islets. Cyclin D2 overexpression rescued the inhibition of glucose-mediated β-cell proliferation induced by KD-PKCζ. This suggests that cyclin D2 is downstream of PKCζ in the glucose-elicited β-cell replication. KD-PKCζ also attenuated glucose-induced phosphorylation of the mechanistic targets of rapamycin (mTOR), ribosomal protein S6 kinase beta-1 (p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1). Inhibition of mTOR with rapamycin reduced glucose-induced cyclin D2 expression in the INS-1 cells. KD-PKCζ had no effect on phosphorylation of Akt or glycogen synthase kinase-3β (GSK-3β). Based on these results, they concluded that PKCζ regulates cyclin D2 expression through mTOR activation, independent of Akt, in the β-cells[3]. However, it is possible that phosphorylation of PKCζ is downstream of Akt and/or GSK-3β, and that the alteration of protein synthesis, mitochondrial functions or autophagy by rapamycin could influence the activity of PKCζ. Glucose- and HFD-induced β-cell proliferation is mediated by insulin receptor substrate-2 (IRS-2)[1,4]. Glucose-induced IRS-2 upregulation is thought to be mediated through the cyclic adenosine monophosphate response element binding protein or calcium/calmodulin-dependent phosphatase calcineurin/nuclear factor of activated T-cells signaling[1,5]. This glucose-IRS2 pathway is also reportedly mediated through mTOR and cyclin D2[4]. Taken together,

glucose and HFD feeding stimulates IRS-2/PI3K signaling through calcineurin or cyclic adenosine monophosphate response element binding protein, followed by activation of PKCζ and mTOR-mediated cyclin D2 expression in the β-cells (Figure 1).

As glucokinase-mediated glucose signaling is required for adaptive β-cell proliferation under the condition of HFD-induced obesity[1], glucose is a prominent mitogenic component for the β-cells.

Insulin treatment also induces phosphorylation of PKCζ, which promotes mTOR activation and β-cell proliferation[3]. However, the contribution of insulin in the adaptive β-cell proliferation under the condition of HFD-induced obesity is still controversial. β-Cell-specific insulin receptor-knockout mice failed to show adaptive β-cell hyperplasia in response to HFD feeding and showed nuclear localization of the forkhead box O1, with reduction in the pancreatic and
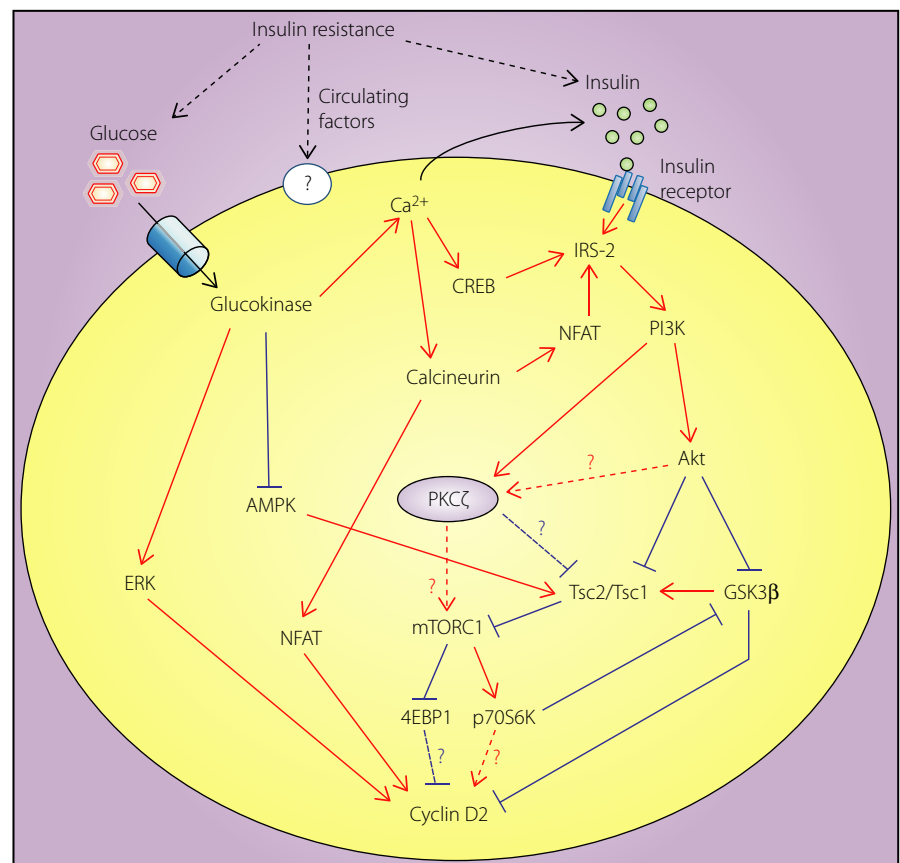

Figure 1 | Schematic representation of the compensatory β-cell proliferative signaling network mediated by insulin resistance. Insulin resistance stimulates β-cell replication through glucose, insulin or other factors, including circulating molecules secreted by other tissues. Glucose-induced β-cell replication is dependent on insulin receptor substrate-2 (IRS-2). The role of insulin/insulin receptor signaling on the ability of β-cells to replicate in response to insulin resistance remains controversial. Activation of protein kinase Cζ (PKCζ) requires phosphoinositide 3-kinase (PI3K) activity; however, the precise mechanism remains unclear. Mechanistic targets of rapamycin complex 1 (mTORC1) activity has also been associated with adenosine monophosphate-activated protein kinase (AMPK), Akt, glycogen synthase kinase-3β (GSK-3β) and feedback signaling of p70S6K. The potential role of PKCζ in the induction of the mTORC1 pathway and cyclin D2 expression requires further investigation. 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; CREB, cyclic adenosine monophosphate response element binding protein; ERK, extracellular signal-regulated kinases; NFAT, nuclear factor of activated T-cells; p70S6K, ribosomal protein S6 kinase beta-1; Tsc2/Tsc1, tuberous sclerosis complex 2/1.

duodenal expressions of homeobox-1[6]. This phenotype is similar to that of β-cell-specific Gck-heterozygous-knockout mice reared on a HFD, which showed an increased percentage of nuclear forkhead box O1-positive β-cells[1]. Furthermore, overexpression of IRS-2 induced forkhead box O1 nuclear export in HFD-fed Gck-heterozygous-knockout mice[1]. However, according to the report from Stamateris et al.[4], insulin receptor stimulation is not sufficient for β-cell replication. They showed that both glucose and insulin induced phosphorylation of p70S6K, 4EBP and Akt as an acute response, at 2–15 min. However, after chronic stimulation for 72 h, glucose stimulated p70S6K and 4EBP1, but not Akt, suggesting that mTOR was activated by glucose stimulation. On the contrary, insulin upregulated Akt, but could not induce p70S6K and 4EBP1 phosphorylation. Stamateris et al.[4] also showed that insulin receptor and extracellular signal-regulated kinases were not necessary for glucose-induced β-cell proliferation, by using inhibitors or short hairpin ribonucleic acid (shRNA) knockdown. It might be interesting to examine PKCζ expression and phosphorylation in the β-cells of HFD-fed β-cell-specific insulin receptor-knockout or Gck-heterozygous-knockout mice. High concentrations of insulin can cross-react with insulin-like growth factor-1 receptor expressed on β-cells. Because there is a functional redundancy between the signals transduced by the insulin-like growth factor-1 receptor and insulin receptor, the insulin-like growth factor-1 receptor-mediated pathway might be involved in PKCζ-mediated β-cell replication under the HFD-induced hyperinsulinemic state. Further studies are required to confirm the role of insulin and PKCζ in adaptive β-cell replication induced by HFD.

PKCζ might be promising for inducing physiological adaptive β-cell replication and serve as a therapeutic target to design means for β-cell mass expansion in diabetes patients. However, we need to clarify whether PKCζ can actually amplify the 'functional' β-cell mass under diabetic conditions. Both β-cell-specific KD-PKCζ-transgenic mice and β-cell-specific PKCζ-knockout mice showed normal glucose and insulin homeostasis after receiving a HFD for a week[3]. The contribution of PKCζ to compensatory β-cell hyperplasia has not yet been precisely assessed. A study involving prolonged HFD feeding with apparent obesity and insulin resistance would be required to evaluate the significance of PKCζ as a regulator of the adaptive response of β-cells. Furthermore, the involvement of PKCζ in the adaptive β-cell mass expansion in response to other physiological demands, such as pregnancy or aging, is unclear. Although rat-insulin promoter-driven PKCζ-transgenic mice showed β-cell proliferation and β-cell mass expansion[7], the impact of PKCζ on the regulation of the β-cell mass under the condition of diabetes (e.g., diabetes model mice) is unclear. Studies are warranted to explore whether PKCζ has an effect on apoptosis, neogenesis or transdifferentiation of β-cells. Of considerable interest, the expression level of cyclin D2, a downstream molecule to PKCζ, is extremely low in human β-cells. However, PKCζ-transgenic mice also showed increased expressions of cyclin D1, cyclin D3 and cyclin A in the islets[7]. Perhaps most importantly, study of the role of PKCζ in human β-cells is indispensable for paving the way for more relevant clinical investigations.

ACKNOWLEDGMENT

DISCLOSURE

The authors declare no conflict of interest.

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