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REVIEW

Signaling Molecules Involved in Lipid-Induced Pancreatic Beta-Cell Dysfunction

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The increasing incidence of type 2 diabetes mellitus is partially due to the rising obesity rates and the elevated levels of free fatty acids (FFAs). It is known that FFAs are putative mediators of beta-cell dysfunction, which is characterized with impaired glucose-stimulated insulin secretion and increased apoptosis, being defined as lipotoxicity. To date, many factors and their related signal pathways have been reported to be involved in FFA-induced beta-cell dysfunction. However, the entire blueprint is still not obtained. Some essential and newfound effectors, including the sterol regulatory element-binding protein (SREBP)-1c, farnesoid X receptor (FXR), forkhead box-containing protein O (FoxO) 1, ubiquitin C-terminal hydrolase L (UCHL) 1, N-myc downstream-regulated gene (NDRG) 2, perilipin family proteins, silent information regulator 2 protein 1 (Sirt1), pituitary adenylate cyclase-activating polypeptide (PACAP), and ghrelin are described in this review, which may help to further understand the molecular network for lipotoxicity.

Introduction

To date, diabetes in all its forms afflicts at least 200 million people in the world and this number is substantially increasing, which is expected to double by the year 2025 (Meetoo *et al.*, 2007). Type 2 diabetes mellitus (T2DM), as a common subtype of diabetes, involves two core defects, that is, insulin resistance and beta-cell dysfunction (Meetoo *et al.*, 2007; Giacca *et al.*, 2011; Zitkus, 2012). It is known that obesity is a major risk factor of T2DM, in part due to elevated circulating free fatty acids (FFAs) (Giacca *et al.*, 2011). Chronic lipid accumulation plays an essential role in pancreatic beta-cell dysfunction characterized with impaired glucose-stimulated insulin secretion (GSIS) and increased levels of apoptosis, being recognized as lipotoxicity (Shao *et al.*, 2010; Giacca *et al.*, 2011). However, the mechanisms of FFA-induced GSIS impairment and lipoapoptosis are not fully understood and the entire blueprint is still under investigation. Some pertinent signaling molecules and their related signal pathways are identified, including sterol regulatory element-binding protein (SREBP)-1c, farnesoid X receptor (FXR), forkhead box-containing protein O (FoxO) 1, ubiquitin C-terminal hydrolase L (UCHL) 1, N-myc downstream-regulated gene (NDRG) 2, perilipin family proteins, silent information regulator 2 protein 1 (Sirt1), pituitary adenylate cyclase-activating polypeptide (PACAP), and ghrelin (Table 1). This review summarized these essential factors and analyzed their crosslinking in an integral view, thus providing a clearer understanding. This could contribute not only to identify the mechanism of beta-cell dysfunction under lipid stress, but also to develop the treatment strategies with new perspectives for T2DM.

Sterol Regulatory Element-Binding Protein-1c

SREBP-1c, a member of the membrane-bound transcription factor basic helix-loop-helix leucine zipper family, is conventionally viewed as a nutritional regulator of lipogenic enzymes in the liver (Shao *et al.*, 2009; Hong *et al.*, 2012). It was upregulated by dietary intake of carbohydrates, sugars, and saturated fatty acids. This type of nutritional SREBP-1c regulation has recently been observed in both cultured beta cells and isolated islets of mice (Kato *et al.*, 2008; Shao *et al.*, 2009). Moreover, transgenic mice overexpressing the active form of SREBP-1c exhibited impaired glucose tolerance *in vivo*, indicating that activation of SREBP-1c could cause beta-cell dysfunction (Takahashi *et al.*, 2005).

Insulin secretion in pancreatic islets was found to be impaired by the addition of palmitate, a typical saturated fatty acid, and this effect was abolished in SREBP-1c-null islets (Takahashi *et al.*, 2005; Kato *et al.*, 2008; Hong *et al.*, 2012). These findings established an association between SREBP-1c and lipid-induced beta-cell dysfunction. Furthermore, palmitate was found to upregulate the expression level of SREBP-1c along with the downregulation of pancreatic and duodenal homeobox (Pdx)-1 and glucagon-like peptide-1 receptor (GLP-1R), two essential effectors for the beta-cell function, in INS-1 cells (Shao *et al.*, 2009, 2010). Additionally, either SREBP-1c ablation or Pdx-1 overexpression could partially alleviate palmitate-induced GSIS impairment, suggesting that sequent

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Table 1. Effectors Involved in Lipotoxicity and Their Established Functions

Effectors involved in beta-cell function	*Abbreviation*	*Function*
Sterol regulatory element-binding protein-1c	SREBP-1c	Transcription factor regulating multiple pathways required for beta-cell function
Farnesoid X receptor	FXR	Transcription factor required for beta-cell function
Forkhead box-containing protein O1	FoxO1	Transcription factor involved in beta-cell survival and GSIS in PI3K/Akt pathway
Ubiquitin C-terminal hydrolaseL1	UCHL1	Deubiquitinating enzyme essential for beta-cell function and survival
N-myc downstream-regulated gene 2	NDRG2	Substrate of Akt involved in the Akt-mediated beta-cell survival
Perilipin Adipocyte differentiation-related protein	Perilipin ADFP	Protein associated to lipid storage
Silent information regulator 2 protein 1	Sirt1	Protein of sirtuin family involved in fat metabolism and GSIS
Pituitary adenylate cyclase-activating polypeptide	PACAP	Peptide of incretin family involved in beta-cell function
Ghrelin	Ghrelin	Peptide secreted from gastric fundus involved insulin secretion and survival

GSIS, glucose stimulated insulin secretion; PI3K, phosphatidylinositol-3 kinase.

SREBP-1c-Pdx-1-GLP-1R signal pathway was involved in the lipid caused GSIS impairment (Shao *et al.*, 2009, 2010) (Fig. 1).

Besides Pdx-1, SREBP-1c, as a transcription factor, was also reported to regulate the expression of genes involved in the beta-cell function, including uncoupling protein (UCP) 2, a protein mediating the energy dissipation in mitochondria instead of ATP synthesis, insulin receptor substrate (IRS)-2, which was involved in the insulin/insulin-like growth factor-1 pathway, GTPase granuphilin related to insulin granules transport for exocytosis, and electrophysiological function-related factors (Kir6.2, Kv1.2, Syntaxin-1a, and Munc18-1) (Kato *et al.*, 2008; Shao *et al.*, 2009, 2010). However, it was unclear whether these effectors are associated with SREBP-1c-involved lipotoxicity (Fig. 2).

Together, the findings reveal that lipid stress could increase the expression level of SREBP-1c. SREBP-1c-Pdx-1-GLP-1R may be one of the signal pathways involved in the lipid-caused GSIS impairment. Various beta-cell function-related

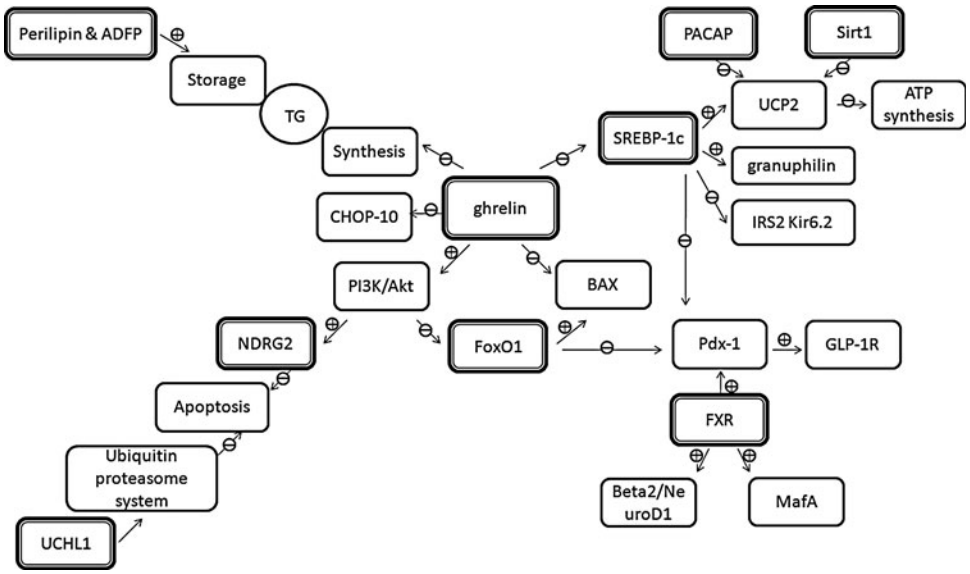

FIG. 1. Schematic diagram of effector intercrossing network under physiological condition. Sterol regulatory element-binding protein (SREBP)-1c is involved in multiple gene regulation, including insulin receptor substrate (IRS)-2, pancreatic and duodenal homeobox (Pdx-1), granuphilin, uncoupling protein (UCP) 2, and ion channels. Pituitary adenylate cyclase-activating polypeptide (PACAP) and silent information regulator 2 protein 1 (Sirt1) could regulate the expression of UCP2 as well. Farnesoid X receptor (FXR) upregulates the expression of Pdx-1, NeuroD1, and MafA. Forkhead box-containing protein O1 (FoxO1) is the downstream mediator of phosphatidylinositol-3 kinase (PI3K)/Akt pathway, which could modulate the activity and/or expression of Pdx-1 and BAX. N-myc downstream-regulated gene (NDRG2) (substrate of Akt) and deubiquitinating enzyme ubiquitin C-terminal hydrolase L (UCHL1) are both involved in beta-cell survival. Perilipin and adipocyte differentiation-related protein (ADFP) can regulate the triglycerides (TG) storage in beta cells. The function of ghrelin includes the activation of PI3K/Akt pathway, the nuclear exclusion of FoxO1, the reduction in cytoplasmic TG synthesis, and the downregulation of BAX, SREBP-1c, and CHOP-10. Frame in bold line, direct target of lipid stress; ⊕, enhance; ⊖, inhibit.

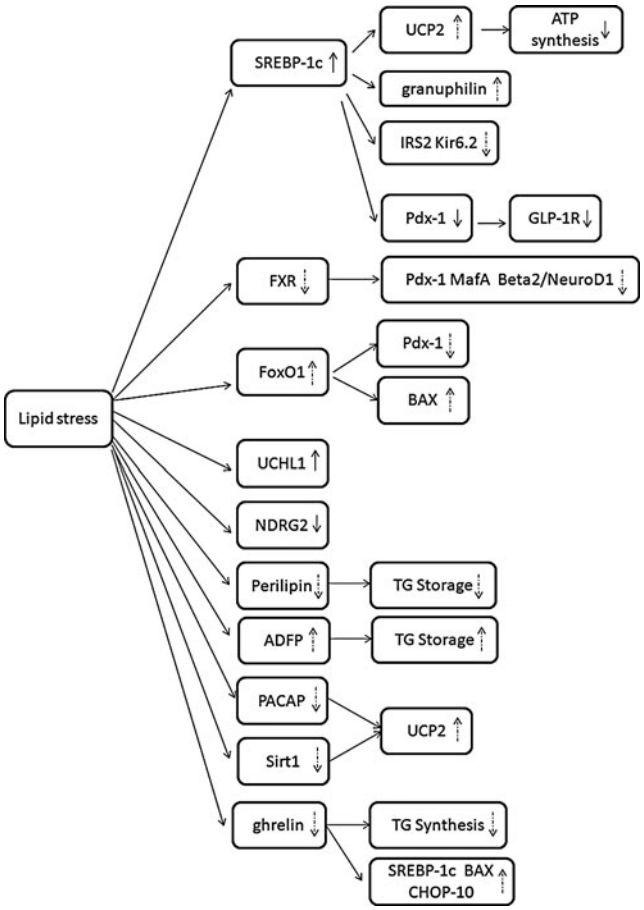

FIG. 2. Expression/activity of effectors under lipotoxic conditions. Lipid stress elevates the expression/activity of SREBP-1c and UCHL1, but downregulates the activity of NDRG2. The regulation of FXR, FoxO1, perilipin, ADFP, PACAP, Sirt1, and ghrelin under lipid stress needs to be further verified. Solid arrows stand for events that have been confirmed and dashed arrows for the events requiring substantiation.

molecules, including UCP2, IRS-2, granuphilin, and Kir6.2 are regulated by SREBP-1c. Nevertheless, whether they are the downstream mediators in the high FFA-SREBP-1c pathway remains to be further identified.

Farnesoid X Receptor

The FXR, as a transcription factor, was highly expressed in liver, intestine, and adrenal glands, where it controlled bile acids, lipid, and glucose homeostasis (Makishima *et al.*, 1999; Popescu *et al.*, 2010; Dufer *et al.*, 2012). It has been found that FXR gene expression was induced by glucose in rat hepatocytes, whereas insulin reversed this effect (Lefebvre *et al.*, 2009). Moreover, *FXR* mRNA levels were increased in livers of diabetic db/db mice (Zhang *et al.*, 2006). In addition, $FXR^{-/-}$ mice displayed impaired glucose and insulin tolerance due to blunted insulin signaling pathways in the skeletal muscle and white adipose tissue (Ma *et al.*, 2006; Popescu *et al.*, 2010).

Recent data demonstrated that FXR was also expressed and functional in pancreatic islets and beta-cell lines, both in rodents and humans (Popescu *et al.*, 2010). Interestingly, FXR was predominantly cytosolic localized in the islets of lean mice, but nuclear in obese mice. Therefore, it is speculated that the lipid stress could lead to the translocation of FXR from nuclear to cytoplasm, resulting in the inactivation of FXR. Further investigation is required to identify this (Fig. 2).

Furthermore, Popescu *et al.* (2010) found that treatment of human islets with FXR agonists protected islets from palmitate-induced triglycerides (TG) accumulation, indicating the protective role of FXR on human islets under lipid-induced metabolic stress. In addition, GSIS was found to be impaired in islets isolated from $FXR^{-/-}$ mice (Popescu *et al.*, 2010). V-maf musculoaponeurotic fibrosarcoma oncogene homolog (Maf) A, a master transcription factor regulating the insulin gene and GSIS in the mature beta cell (Shao *et al.*, 2009), was decreased in $FXR^{-/-}$ pancreas. Besides, Beta2/NeuroD1, a key transcription factor of islet cells (Shao *et al.*, 2009), in addition to Pdx-1 were also reduced in $FXR^{-/-}$ pancreas (Popescu *et al.*, 2010) (Fig. 1). Whether these targets are involved in the lipid stress–FXR signal pathway requires further exploration.

In conclusion, the identification of FXR in the control of beta-cell function opens promising new notions for the prevention of lipotoxicity. Nevertheless, the function of FXR under physiological conditions is still not thoroughly identified although its protective effect on the beta-cell function is found in $FXR^{-/-}$ mice. In addition, the downstream mediators in the high FFAs-FXR pathway are not completely obvious. Further research is demanded.

Forkhead Box-Containing Protein O1

Forkhead box-containing O (FoxO) proteins (FoxO1, FoxO3, FoxO4, and FoxO6) are a subclass of the large family of forkhead proteins featured by the presence of a winged helix DNA-binding domain called the forkhead box (Kaestner *et al.*, 2000). They are important for cellular differentiation, proliferation, apoptosis, and stress resistance. FoxO1, the most abundant isoform in liver, adipose tissue, and beta cells, is conventionally viewed as a regulator in glucose and lipid production in liver (Kitamura *et al.*, 2002). In addition, FoxO1 was found to suppress the expression of genes related to lipogenesis, including *SREBP-1c* in liver (Zhang *et al.*, 2006) and stimulate fatty acid uptake and utilization in muscle (Bastie *et al.*, 2005).

It is known that a high FFA load, when exceeding beta-cell esterification capacity, might impair endoplasmic reticulum (ER) functions and trigger an ER stress response, contributing to beta-cell toxicity (Martinez *et al.*, 2008; Giacca *et al.*, 2011). Inhibition of FoxO1 decreased the expression of the ER stress marker gene and consequently promoted beta-cell survival under FFA stress, suggesting the effect of FoxO1 on lipid-induced beta-cell apoptosis (Martinez *et al.*, 2008). Further investigation demonstrated that, as a prominent downstream target of FoxO1, the proapoptotic BCL-2 family member BAX mediated the effect of FoxO1 on lipoapoptosis (Kim *et al.*, 2005) (Fig. 1). More recently, transgenic mice with constitutively active FoxO1 in the pancreas presented impaired glucose tolerance in addition to the reduction of beta-cell mass (Kikuchi *et al.*, 2012). On the contrary, FoxO1 ablation in beta cells resulted in enhanced insulin secretion at

high glucose concentrations, indicating the role of FoxO1 on GSIS (Miyazaki *et al.*, 2012). Whether such negative effects of FoxO1 on insulin secretion act under lipid stress requires further exploration.

Additionally, Pdx-1 was found to be regulated by another forkhead transcription factor FoxA2 (Lee *et al.*, 2002). FoxO1 and FoxA2 shared common DNA-binding sites in the Pdx-1 promoter. Thus, FoxO1 may inhibit the transcription of Pdx-1 through competing the binding sites with FoxA2 (Fig. 1). Furthermore, transgenic mice with FoxO1 overexpression in the pancreas developed diabetes with the decreased expression of Pdx1 in islets, which further demonstrated the inhibitory role of FoxO1 on Pdx-1expression (Kikuchi *et al.*, 2012).

Besides, evidence has indicated that activation of the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (PKB, also known as Akt) pathway prevented palmitate-induced toxicity of pancreatic beta cells (Granata *et al.*, 2007). Recent studies further identified that FoxO1 was an important mediator in the PI3K/Akt pathway, which could be phosphorylated on Thr24, Ser256, and Ser316 by Akt, resulting in transport of FoxO1 from the nucleus to the cytoplasm (Kim *et al.*, 2005; Fang *et al.*, 2012) (Fig. 1). When FoxO1 was inhibited, pancreatic beta cells were protected against FFA-induced apoptosis (Martinez *et al.*, 2008). These findings indicated the essential role of the PI3K/Akt/FoxO1 pathway in beta-cell lipoapoptosis.

Taken together, the PI3K/Akt/FoxO1 pathway may be of importance in beta-cell lipoapoptosis and GSIS impairment. Stronger clues are required for the direct correlation between lipid stress and FoxO1 activation (Fig. 2). Moreover, negative regulation of Pdx-1 by FoxO1 may also contribute to the development of lipotoxicity.

Ubiquitin C-Terminal Hydrolase L 1

Ubiquitination, an important reversible post-translational modification, can target proteins for degradation or modify their activity (Hershko *et al.*, 2000). An emerging clue implies essential roles for ubiquitination in both beta-cell survival and insulin secretion.

UCHL1 was first identified as a deubiquitinating enzyme that hydrolyzed the peptide bond at the C terminus of ubiquitin and was involved in the processing of ubiquitin precursors and the polyubiquitin chains (Nijman *et al.*, 2005). Additionally, UCHL1 could stabilize free ubiquitin and prevent its degradation (Nijman *et al.*, 2005). The expression of UCHL1 was enriched in the brain, testis/ovary, and pancreatic islets (Lopez-Avalos *et al.*, 2006). Mutations and modifications of UCHL1 were associated with human neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (Choi *et al.*, 2004).

Recently, emerging evidence pointed to the importance of UCHL1 in pancreatic beta cells (Lopez-Avalos *et al.*, 2006). UCHL1 was identified in a proteomic screen as the most upregulated protein in MIN6 beta cells treated with palmitate (Chu *et al.*, 2012). In this study, Chu *et al.* used a genetic loss-of-function model to test the hypothesis that UCHL1 was required for normal beta-cell function and fate under lipotoxic conditions and it was found that a 4-week high-fat (HFa) diet caused glucose intolerance and impaired insulin secretion in *UCHL1*$^{-/-}$ mice. In addition, increased ER stress and beta-cell apoptosis were observed in *UCHL1*$^{-/-}$ mice as well (Chu *et al.*, 2012).

These data suggest that UCHL1 has essential functional and antiapoptotic roles in beta cells under lipid stress, highlighting a novel understanding of the importance of UCHL1 and the ubiquitin proteasome system in the pathobiology of lipotoxicity and T2DM.

N-myc Downstream-Regulated Gene 2

Human *NDRG2* was first cloned from a human brain cDNA library by subtractive hybridization (Lachat *et al.*, 2002). NDRG2, as a member of the NDRG gene family, played a variety of roles in cell proliferation and differentiation, stress response, and p53- and HIF-1-mediated apoptosis (Lachat *et al.*, 2002). Several reports have provided insight into the physiological roles of NDRG2 in the central nervous system (Shen *et al.*, 2008). In addition to its known functions in the brain, NDRG2 acted as a novel regulator for myoblast proliferation and was regarded as a putative tumor suppressor in human cancer (Foletta *et al.*, 2009; Shen *et al.*, 2010). Absence of NDRG2 may influent cell apoptosis under specific stress conditions (Foletta *et al.*, 2009).

However, the function of NDRG2 in the pancreas remains to be established. Hu *et al.* (2006) demonstrated NDRG2 immunoreactivity in mouse islets. Further investigation revealed the strong NDRG2 expression in pancreatic beta cells, suggesting a potential role of NDRG2 in beta-cell function (Shen *et al.*, 2010). To expand earlier observations, Shen and colleague investigated the biological functions of NDRG2 and found that NDRG2 was a potential substrate of protein kinase Akt (Shen *et al.*, 2010). Akt, an important molecule in the insulin signaling pathway, could promote the survival of pancreatic islets and prevent beta-cell apoptosis induced by FFAs (Granata *et al.*, 2007). In Shen's study, when clonal beta-cell line b-TC3 cells were exposed chronically to high levels of FFAs, cell viability was impaired along with the reduced phosphorylation of Akt and NDRG2 (Shen *et al.*, 2010). In addition, the overexpression of constitutively active Akt enhanced NDRG2 phosphorylation and abolished the apoptosis induced by FFAs in b-TC3 cells, whereas NDRG2 knockdown attenuated Akt-mediated protection of beta cells against lipoapoptosis (Shen *et al.*, 2010).

Collectively, these data indicate that NDRG2 is involved in the Akt-mediated protection of beta cells against lipotoxicity and functions as a key molecule in beta-cell survival. The findings may represent a novel area for therapeutic intervention in lipid stress. However, the downstream pathway of Akt-NDRG2 remains to be identified as well.

Perilipin Family

Lipid droplets (LDs), as dynamic functional organelles, is of importance in cellular energy balance, the structure of which contains a core of neutral lipid (TGs and cholesterol ester) coated by an interface composed of a monolayer of phospholipids, free cholesterol, and proteins (Ducharme *et al.*, 2008). The storage droplets may help transport the neutral lipids to specific cellular destinations or direct them to specific metabolic or signaling pathways. Such coordination of lipid metabolism was likely controlled by the LD coat proteins of the perilipin family, including the founding member perilipin, as well as the adipocyte differentiation-related

protein (ADFP), tail-interacting protein of 47 kilodaltons (TIP47), S3–12, and oxidative tissue-enriched PAT protein (OXPAT) (Miura *et al.*, 2002). These perilipin family proteins shared sequence similarity and localized to LDs, either constitutively (perilipin, ADFP) or in response to lipogenic stimuli (TIP47, S3–12, and OXPAT) (Brasaemle *et al.*, 2007).

Perilipin, the most highly studied member of the perilipin family, played an important role in the regulation of basal and hormonally stimulated lipolysis (Forcheron *et al.*, 2005; Mishra *et al.*, 2005; Borg *et al.*, 2009). It has been known that the excess accumulation of lipids in islets contribute to the development of T2DM in obesity by the impairment of beta-cell function. Furthermore, an emerging perspective indicates that sequestration of cytosolic FFAs in cellular TG stores represents a cytoprotective mechanism for lipotoxicity in beta cells. Borg *et al.* (2009) provided evidences for expression of perilipin in rat, mouse, and human islets as well as the rat clonal beta-cell line INS-1 at low levels and showed that overexpression of perilipin increased the capability of lipid storage in beta cells (Fig. 1). To examine whether the development of lipotoxicity could be prevented by manipulating the conditions for lipid storage, INS-1 cells with perilipin overexpression were exposed to lipotoxic conditions and the GSIS was retained, indicating that overexpression of perilipin appeared to confer a protective effect against FFA load. This may be attributed to the expansion of lipid storage in beta cells (Borg *et al.*, 2009) (Fig. 2). However, it was still not clear whether the high level of FFAs would affect the expression of perilipin. Identifying this should be of significance.

Besides perilipin, ADFP is also localized on the surface of LDs in a wide range of cells with islets included, and serves crucial functions in intracellular lipid metabolism. The upregulation of ADFP was found at the site of increased lipid accumulation, such as fatty liver (Heid *et al.*, 1998). Moreover, Imai *et al.* reported that the reduction of ADFP via antisense oligonucleotide (ASO) could reverse fatty liver and alter lipid metabolism (Imai *et al.*, 2007). More recently, it was found that the HFa diet could markedly increase the expression level of ADFP in murine islets along with the incremental expression of ADFP in human islets by addition of FFAs (Faleck *et al.*, 2010). Effect of long-term lipotoxicity on the expression/activity of ADFP in beta cells remains to be identified (Fig. 2). Furthermore, the downregulation of ADFP in MIN6 cells by ASO resulted in the suppression of the TG accumulation upon FFAs loading, indicating that ADFP may participate in the regulation of intracellular lipid metabolism in islet beta cells (Faleck *et al.*, 2010) (Fig. 1). However, few studies focused on the association of ADFP and beta-cell function under lipid stress. Future investigations should address whether ADFP has a unique role in the prevention of lipotoxicity in islets, and it is plausible that ADFP has a distinct position in the development of lipotoxic-induced beta-cell dysfunction as a LD protein.

Taken together, these results indicate a new concept to abolish the lipotoxic effects of chronic exposure to FFAs by increasing the capacity of beta cells for efficiently storing excess FFAs. Further studies of perilipin and ADFP will increase our understanding of lipid metabolism in pancreatic islets, which is critical in targeting lipotoxicity. Roles of other members of the perilipin family in lipotoxic-induced beta-cell dysfunction should be further explored.

Silent Information Regulator 2 Protein 1

Sirtuins (Sirt) belong to a highly conserved family of protein deacetylases and ADP-ribosyltransferases with seven members (Sirt1–7) in mammals (Haigis and Guarente, 2006). Sirt1, the best studied sirtuin, is expressed throughout the body, including the brain, heart, liver, pancreas, skeletal muscle, spleen, and adipose tissues. It is known that Sirt1 plays an essential role in multiple biological processes encompassing metabolism, oxidative stress, cellular proliferation, cellular aging, endothelial functions, and genomic stability (Haigis and Guarente, 2006; Chong *et al.*, 2012).

Sirt1 is now considered to be closely connected to the development of T2DM in virtue of its activity on insulin sensitivity. In insulin-resistant cells, the Sirt1 protein was markedly decreased and the reduction of Sirt1 levels in the gastrocnemius muscle in mice resulted in glucose intolerance (Sun *et al.*, 2007). In contrast, overexpression of Sirt1 in the liver served to attenuate hepatic steatosis and improved insulin sensitivity, resulting in ameliorative glucose homeostasis (Li *et al.*, 2011).

Moreover, Sirt1 not only had a role in insulin sensitivity, but also could affect fat metabolism and obesity. In obese mice, Sirt1 expression was low in adipose tissue and loss of Sirt1 in white adipose cells resulted in the impairment of fatty acid mobilization (Picard *et al.*, 2004). Recent reports found that Sirt1 levels were increased in fat tissues in response to fasting and calorie restriction (CR) in rodents (Cohen *et al.*, 2004; Al-Regaiey *et al.*, 2005). In addition, Chen *et al.* (2010) found that treatment with the CR diet in rats increased the expression of Sirt1 in the pancreatic beta cells, while treatment with the HFa diet caused a decrease. Therefore, it is speculated that lipid stress may regulate the expression/activity of Sirt1 in beta cells (Fig. 2).

Besides, recent evidence demonstrated that the improvement of GSIS in INS-1 cells and human islets by the Sirt1 activator resveratrol was dependent on active Sirt1 (Vetterli *et al.*, 2011). Furthermore, it was found that Sirt1 could promote insulin secretion in pancreatic beta cells in response to glucose, partly, through the repression of UCP2 (Moynihan *et al.*, 2005; Bordone *et al.*, 2006; Ramsey *et al.*, 2008) (Fig. 1). Pancreatic beta cell-specific Sirt1-overexpressing (BESTO) transgenic mice exhibited enhanced GSIS and improved glucose tolerance (Moynihan *et al.*, 2005). Additionally, BESTO islets showed reduced levels of UCP2 and correspondingly increased levels of ATP. Consistent with the results from BESTO mice, insulin secretion was impaired along with the increased level of UCP2 in whole-body $Sirt1^{-/-}$ mice, where Sirt1 was knocked down by RNA interference (Bordone *et al.*, 2006). Evidence from BESTO and $Sirt1^{-/-}$ mice suggests that Sirt1 promotes insulin secretion by repressing the expression of UCP2.

Taken together, Sirt1 has been implicated in numerous metabolic pathways, including adipogenesis and insulin secretion. In particular, CR/HFa diets could regulate the expression of Sirt1 in beta cells, suggesting that Sirt1 activation may have some positive actions in lipotoxicity. Direct evidence is required to uncover it and ultimately provide a clearer perspective. Furthermore, it has been identified that the beneficial effect of Sirt1 on beta-cell function is attributed to the repression of UCP2. However, it is unclear whether this effect could play a part under the lipotoxic condition in T2DM. In addition, further studies are needed to identify

other target molecules regulated by Sirt1 in addition to UCP2. Such challenges must be met to provide novel and urgently needed therapeutic strategies for the treatment of T2DM, and to prevent the associated lipid stress.

Pituitary Adenylate Cyclase-Activating Polypeptide

PACAP, a peptide of incretin family, is known to be located in the central and peripheral nervous systems. Recent data demonstrated that it located in pancreatic islets as well and could potentiate GSIS as an effective insulinotropin in an autocrine and/or paracrine manner (Filipsson *et al.*, 1997; Sakurai *et al.*, 2011). Further studies found that PACAP could be synthesized by islets and stored in the secretory granules of both beta cells and alpha cells (Nakata and Yada, 2007). The insulinotropic action involved the binding of PACAP to its specific receptors and the successive coupling to both cyclic AMP and Ca^{2+} signaling, the pathways known to be employed also by other incretin hormones, such as GLP-1 (Nakata and Yada, 2007; Sakurai *et al.*, 2011). In addition, transgenic mice with beta-cell-specific overexpression of PACAP were resistant to streptozotocin-induced beta-cell destruction, suggesting the protective effect of PACAP for islet beta cells (Yamamoto *et al.*, 2003).

In the study from Nakata, short-term treatment of islets with palmitate was performed and it was found that the treatment markedly impaired both $[Ca^{2+}]i$ and GSIS in islets of PACAP-null, but not wild-type mice (Nakata *et al.*, 2010). These results indicated that endogenous PACAP in islets played an important role in protecting beta cells against lipotoxicity. Moreover, treatment with palmitate also obviously increased the expression of UCP2 mRNA in islets of PACAP-null compared with wild-type mice, suggesting that PACAP exerted its protective effect, at least partly, via counteracting the elevation of UCP2 expression (Fig. 1). Nevertheless, all these findings were under the experimental manipulation. Whether the synthesis and activity of PACAP are affected by lipotoxic condition remain to be researched. In addition, the downstream effectors in the PACAP pathway need to be expanded.

Collectively, islet-produced PACAP could protect beta cells from lipotoxicity, indicating a potential antidiabetic role for PACAP to prevent and/or treat lipid-induced beta-cell dysfunction.

Ghrelin

Ghrelin, a 28-amino-acid peptide, is secreted predominantly from X/A-like cells of the gastric fundus. Through the orphan growth hormone secretagogue receptor type 1a (GHSR1a), ghrelin acted as a growth hormone-releasing peptide to affect and/or modulate energy and glucose homeostasis; gastrointestinal, cardiovascular, pulmonary, and immune functions; and cell proliferation and differentiation (Soares and Leite-Moreira, 2008).

Recently, the effect of ghrelin on pancreatic beta-cell function attracted much attention. Emerging studies have identified ghrelin-secreting cells (epsilon-cells) in the verges of pancreatic islets, suggesting that ghrelin affected beta cells through both the endocrine and paracrine pathways (Prado *et al.*, 2004; Vignjević *et al.*, 2012). Further study reported that ghrelin could stimulate insulin secretion (Granata *et al.*, 2007), although inhibitory effects were also reported (Dezaki *et al.*, 2004; Sun *et al.*, 2006; Tong *et al.*, 2010). In addition, Bando *et al.* (2012) developed a transgenic mouse model in which the ghrelin genes were overexpressed; however, such overexpression of intraislet ghrelin did not affect insulin secretion *in vivo*. This controversy may be, at least in part, attributed to physiologic or pharmacologic doses of ghrelin, which necessitates further investigation.

Despite the inconsistent effects regarding the effect of ghrelin on insulin secretion, accumulating evidence supports a role of ghrelin in the regulation of proliferation and differentiation of pancreatic beta cells. It was reported that ghrelin could inhibit beta-cell apoptosis induced by the interferon-r/tumor necrosis factor-a (IFN-r/TNF-a), doxorubicin, and lipid stress (Granata *et al.*, 2007; Zhang *et al.*, 2007; Wang *et al.*, 2010). Furthermore, it was reported that the protective effect of ghrelin against lipid stress may via, at least in part, the PI3K/Akt signaling pathway by increasing beta-cell proliferation and survival (Granata *et al.*, 2007). Additionally, ghrelin also inhibited the FFA-induced ER stress pathway of apoptosis in MIN6 cells, decreased expression of cytoplasmic TG, and downregulated gene expression of Bcl-2-associated X (BAX), SREBP-1c, and C/EBP homologous protein (CHOP-10) (Wang *et al.*, 2010). Furthermore, Wang *et al.* (2010) found that ghrelin inhibited the nuclear translocation of FoxO1 in pancreatic beta cells under lipotoxic pressure, suggesting that the inhibition of FoxO1 may play a key role in the antilipotoxic effect of ghrelin in beta cells.

In summary, the protective action of ghrelin on beta-cell function under lipid stress involves the activation of the PI3K/Akt signaling pathway, the nuclear exclusion of FoxO1, a decrease in cytoplasmic TG synthesis, and the downregulation of BAX, SREBP-1c, and CHOP-10 (Fig. 1).

Conclusion

Lipotoxicity-induced beta-cell dysfunction includes impaired GSIS and increased lipoapoptosis. To date, many effectors have been found to be involved in lipotoxicity (Table 1). It is identified that the lipotoxic condition can elevate the expression/activity of SREBP-1c and UCHL1, but downregulate the activity of NDRG2. Whereas, stronger evidences for the expression/activity of other factors, including FXR, FoxO1, perilipin, ADFP, PACAP, Sirt1, and ghrelin, under lipid stress are necessitated (Fig. 2).

SREBP-1c, FXR, and FoxO1 are the most studied transcription factors for lipotoxicity and now attracting much attention from researchers in this field. Various beta-cell function-related molecules, including Pdx-1, UCP2, IRS-2, granuphilin, and Kir6.2, are known to be regulated by SREBP-1c (Fig. 1). Among these effectors, the Pdx-1 and SREBP-1c-Pdx-1-GLP-1R pathway may be one of essential signal pathways involved in the lipid-induced beta-cell dysfunction. However, whether other effectors mentioned above are involved in the lipotoxicity-SREBP-1c pathway is not quite clear (Fig. 2). FXR can enhance the expression of Pdx-1, NeuroD1, and MafA as shown in Figure 1 under physiological conditions. In addition, the regulation of Pdx-1 and BAX by FoxO1 is identified as well. However, the function of FXR and FoxO1 and their related signaling pathways under lipid stress need further investigation.

The PI3K/Akt pathway is essential for lipid-induced toxicity in pancreatic beta cells. The most studied downstream effector in this pathway is FoxO1. Recent evidences indicate that NDRG2, as a potential substrate of Akt, is involved in the

Akt-mediated protection of beta cells against lipoapoptosis as well. However, the downstream pathway of Akt/NDRG2 is still not completely uncovered. It would be of significance to find out other effectors in the PI3K/Akt signal pathway under lipid stress.

Ghrelin is found to be an important peptide, which is secreted predominantly from the gastric fundus. As shown in Figure 1, it stands in the center of the molecular network related to lipotoxicity. It regulates the activity and/or expression of transcription factors SREBP-1c and FoxO1, the synthesis of cytoplasmic TG, the activity of PI3K/Akt, and the expression of the BCL-2 family member BAX. Other possible targets are required to be identified.

Furthermore, both PACAP and Sirt1 could repress the expression of UCP2, resulting in the increase of ATP synthesis. Moreover, emerging understanding of the importance of UCHL1 and the ubiquitin proteasome system in addition to lipid storage-related proteins (perilipin and ADFP) opens new perspectives in the molecular mechanisms for lipotoxicity. It would be much meaningful to explore other new and possible mechanisms in this field.

To summarize, although substantial work has been done, the entire blueprint related to FFA-induced beta-cell dysfunction is still not obtained. A consistent line connecting all these various molecules remains to be explored. Furthermore, application of these involved effectors into the clinical therapy for T2DM is still in the initial phase. To thoroughly understand the molecular network for lipotoxicity may help to postpone and/or terminate the development of T2DM under lipid stress.

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