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New insight into the mechanisms underlying the function of the incretin hormone glucagon-like peptide-1 in pancreatic β-cells

The involvement of the Wnt signaling pathway effector β-catenin

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During the past two decades, the exploration of function of two incretin hormones, namely glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP), has led to the development of two categories of novel therapeutic agents for diabetes and its complications, known as GLP-1 receptor (GLP-1R) agonists and DPP-IV inhibitors. Mechanisms underlying the function of GLP-1, however, still need to be further explored. GLP-1 not only functions as an incretin hormone in stimulating insulin secretion in response to nutritional, hormonal and neuronal stimulations, but also acts as an "insulin-like" factor in β-cell and extra-pancreatic organs. In addition to these insulinotropic and insulinomimetic effects, GLP-1 was shown to exert its protective effect in β-cell by repressing the expression of TxNIP, a mediator of glucolipotoxicity. A number of recent studies have shown that the Wnt signaling pathway effector, the bipartite transcription factor β-catenin/TCF, controls not only the production of GLP-1, but also the function of GLP-1. Furthermore, previously assumed "degradation" products of GLP-1(7–36)amide, including GLP-1(9–36)amide and GLP-1(28–36)amide, have been shown to exert beneficial effect in pancreas and extra-pancreatic tissues or cell lineages. Here we summarized our current knowledge on the metabolic, proliferative and protective effects of GLP-1(7–36)amide and its cleavage fragments, mainly focusing on pancreatic β-cells and the involvement of the Wnt signaling pathway effector β-catenin.

Introduction

The proglucagon gene (*gcg*) is expressed in pancreatic α-cells, L-type endocrine cells in the gut, and certain neuronal cells in the brainstem.[1,2] The three organs express the same *gcg* cDNA, which encodes the identical pro-hormone known as proglucagon. Tissue specific post-translational processes lead to the generation of different profiles of proglucagon derived peptides, including the three major peptide hormones known as glucagon, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). Glucagon is mainly produced in the pancreas, while the gut produces GLP-1 and GLP-2, but not glucagon. The pancreatic glucagon is a major counter-regulatory hormone of insulin in regulating glucose homeostasis. The gut produced GLP-1, however, is an incretin, exerting an opposite effect with glucagon via stimulating insulin secretion in a glucose concentration dependent manner. GLP-2 is known as a growth factor for small intestinal epithelium.[1,2]

During the past two decades, the exploration of mechanisms underlying the function of GLP-1 and another incretin, gastric inhibitory polypeptide (GIP), has led to the development of two categories of novel therapeutic agents for diabetes and potentially its complications, namely GLP-1 receptor (GLP-1R) agonists and DPP-IV inhibitors.[3,4] Extensive investigations have shown that in addition to functioning as an incretin, GLP-1 possesses "insulin-like" or insulinomimetic effect on pancreatic β-cells and extra-pancreatic tissues or cell lineages. Furthermore, previously assumed "degradation" fragments of GLP-1, namely GLP-1(9–36)amide and GLP-1(28–36)amide, were shown to possess certain beneficial effects in both in vitro and in vivo settings.[5-9] In addition to its insulinotropic and insulinomimetic effects, GLP-1 was also shown to exert protective effects in pancreatic β-cells, exemplified by reducing the levels of TxNIP, a major mediator of glucotoxicity.[10] In exploring mechanisms underlying GLP-1 function, a few recent studies have demonstrated that the Wnt signaling pathway effector cat/TCF, a complex formed by free β-catenin (β-cat) and a member of the T-cell factor (TCF) family, regulates not only gut *gcg* mRNA transcription and GLP-1 production, but also GLP-1 function in pancreatic β-cells and brain neuronal cells.[11-13] Here we present our current knowledge on GLP-1 and its "degradation" products, and summarize our current understanding on the metabolic, proliferative and protective effects of GLP-1. We mainly focus on GLP-1

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and its "degradation" fragments in pancreatic β-cells, as well as the involvement of the Wnt signaling pathway effector β-cat. The involvement of another Wnt pathway effector, the diabetes risk gene TCF7L2, has been summarized elsewhere by our group and by others.[14-17] For the function of GLP-1 in extra-pancreatic organs and tissues, please refer to a large body of excellent recent review articles elsewhere.[7,18]

GLP-1 and its "Degradation" Fragments

GLP-1 is encoded by *gcg* and produced by intestinal endocrine L cells throughout the entire small intestine and colon, with the highest levels within the distal ileum.[19] *gcg* also encodes glucagon, produced in and secreted by pancreatic α-cells. In addition, it encodes GLP-2 in the gut, which functions as a growth factor for the small intestinal epithelium.[20] **Figure 1A** shows the overall structure of the prohormone that is encoded by *gcg* and the cleavage sites of the two prohormone convertases, PC2 and PC1/3. In pancreatic α-cells, the main products of the cleavage include glucagon, glicentin-related pancreatic polypeptide (GRPP), intervening peptide-1 (IP1) and major proglucagon fragment (MPGF) (**Fig. 1A**). During embryonic developmental stages or after pancreatic islets encounter certain stress, small amounts of GLP-1 can be detected in pancreatic α-cells.[21] In both the intestine and the brainstem, the post-translational products include glicentin, GLP-1, GLP-2, GRPP, oxyntomodulin and the intervening peptide-2 (IP2) (**Fig. 1A**). GLP-1(7–37) and GLP-1(7–36)amide are the two biologically active forms of the incretin. For convenience, the term GLP-1(7–36) will be utilized to represent both of them hereafter. The half life of GLP-1(7–36) is very short in circulation. This is due to the presence of the degrading enzymes dipeptidyl peptidase IV (DPP-IV) and neutral endopeptidase NEP24.11 (neprilysin or CD10).[22] The cleavage of GLP-1(7–36) by DPP-IV leads to the generation of GLP-1(9–36), while the cleavage of GLP-1(7–36) or GLP-1(9–36) by NEP24.11 leads to the generation of GLP-1(28–36), the C-terminal nonapeptide FIAWLVKGR (**Fig. 1B**). Although inhibition of either DPP-IV or NEP24.11 has been demonstrated to enhance the incretin effect of GLP-1;[23,24] GLP-1(9–36) and GLP-1(28–36) have been shown to exert beneficial effects on glucose and metabolic homeostasis,[5,7-9,25] indicating that they have the potential to be developed as therapeutic agents for the treatment of diabetes or other metabolic disorders.

Insulinotropic Effect of GLP-1

Postprandial GLP-1 secretion or GLP-1(7–36)/exendin-4 administration leads to a rapid stimulation of insulin secretion, which is mediated through the G-protein coupled receptor GLP-1R in the presence of high levels of glucose. This involves the closure of ATP-sensitive K^+ channels (K_{ATP}), followed by cell membrane depolarization, elevation of the intracellular Ca^{2+} level and Ca^{2+}-induced insulin secretion.[2,26,27] Glucose is required for GLP-1-mediated activation of insulin secretion. Twenty years ago, Holz and colleagues have demonstrated that GLP-1 confers glucose sensitivity to glucose-resistant β-cells, a phenomenon they termed as glucose competence.[28] The α-subunit of the G protein that is coupled by GLP-1R is a Gs, which mediates the activation of adenylyl cyclase (AC), the elevation of cytoplasmic cAMP level and the stimulation of Protein Kinase A (PKA) signaling pathway. In addition, phosphatidylinositol 3-kinase (PI3K)/Protein Kinase B (PKB/Akt), mitogen-activated protein kinase (MEK)/extracellular regulated kinase (ERK), as well as epidermal growth factor receptor (EGFR) signaling can also be activated by GLP-1(7–36).[29] Studies by several laboratories have shown that both PKA and exchange protein activated by cAMP (Epac) signaling pathways are involved in GLP-1 mediated insulin secretion.[26,30-33] Furthermore, GLP-1(7–36) may also stimulate insulin secretion via the inhibition of voltage-dependent K^+ channels.[34]

Insulinomimetic and Protective Effects of GLP-1

The "insulin-like" effect of GLP-1(7–36) was first demonstrated in the stimulation of hepatic glucose uptake in obese human subjects.[35] An ex vivo study with rat hepatocytes indicated that GLP-1(7–36) activates glycogen synthase, possibly via PI3K and Akt signaling cascades.[36] Although an early study with the infusion of GLP-1(9–36) into non-obese individual generated no improvement on insulin release or glucose disposal,[37] a later study in obese and insulin resistance subjects showed a profound inhibitory effect of GLP-1(9–36) on hepatic glucose production.[38] There is still an ongoing debate about the existence of GLP-1R in hepatocytes, and it is not clear whether GLP-1(7–36) and GLP-(9–36) exert their insulinomimetic effect in hepatocytes via a yet to be identified novel receptor, or through a receptor-independent mechanism, or through their further cleaved fragment [such as GLP-1(28–36)].[7]

Both GLP-1(7–36) and GLP-1(9–36) were found to exert insulin-like action in the heart and vasculatures. They improve cardiac function and increase glucose uptake in isolated mouse heart with ischemic myocardial damage.[39] At least some of the beneficial effects by GLP-1(7–36) and GLP-1(9–36) are not mediated by the canonical GLP-1R.[5] A recent study shows that GLP-1(7–36) is able to recruit microvasculature and increases glucose usage in muscle via a nitric oxide-dependent mechanism.[40]

GLP-1(7–36) and the GLP-1R agonist exendin-4 are known to stimulate pancreatic β-cell proliferation, enhance β-cell survival and neogenesis. More recently, a study by Liu and Habeber indicated that the nonapeptide GLP-1(28–36) possesses the cytoprotective effect in the rat pancreatic clonal cell line INS-1. This nonapeptide may target mitochondria and improve mitochondrial membrane potential, elevate cellular ATP level, inhibit cytochrome c mediated cell apoptosis, and enhance the viability and survival of this β-cell line.[25]

Another important protective effect of GLP-1(7–36) was demonstrated in studying a major mediator of glucotoxicity. The thiol oxidoreductase, thioredoxin (TRX) is ubiquitously expressed and participates in maintaining cell redox balance, protecting cells against oxidative stress via scavenging reactive oxygen species. TRX binding protein-2 (TBP-2), also known as thioredoxin-interacting protein (TxNIP), negatively regulates the function of TRX.[41] The intracellular TxNIP level is highly correlated with the blood glucose level.[42] Several investigations have revealed

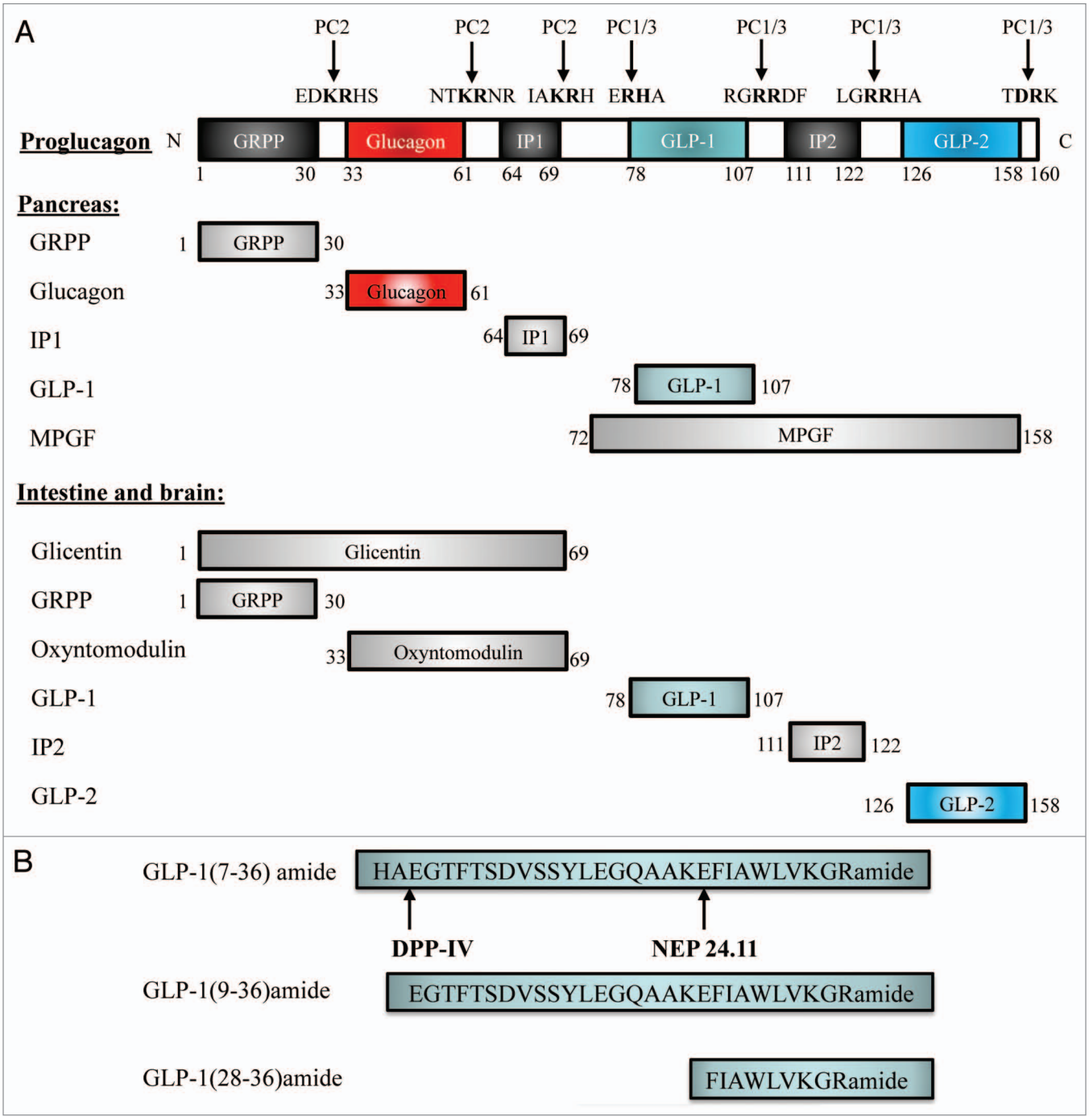

Figure 1. Proglucagon and its cleavage products. (**A**) The *gcg* gene encode proglucagon, a pro-hormone with 160 amino acid residues (top panel). This pro-hormone contains three PC2 and four PC1/3 cleavage sites. A schematic presentation of the cleavage products of proglucagon in the pancreas (middle panel) and in the intestine and brain (bottom panel). (**B**) Amino acid sequences of GLP-1(7–36)amide, GLP-1(9–36)amide and GLP-1(28–36) amide. The cleavage sites for DPP-IV and NEP24.11 are indicated with arrows. GRPP, glycentin related polypeptide; IP1 and IP2, intervening peptide 1 and 2; MPGF, major proglucagon fragment; DPP-IV, dipeptidyl peptidase-4; NEP 24.11, neutral endopeptidase 24.11.

the causative role of TxNIP in β-cell apoptosis, providing a mechanistic link between glucotoxicity, β-cell death, and thus, the progression of type 2 diabetes.[43-46] A few recent studies have shown that exendin-4 is able to reduce TxNIP level in pancreatic β-cells.[42,46] We found that this is at least partially due to cAMP/PKA and cAMP/Epac mediated degradation of TxNIP protein.[10]

Wnt Signaling Pathway and its Effector β-Cat in GLP-1 Production and Function

Introduction of the Wnt signaling pathway and its effector β-cat. The Wnt signaling pathway was initially recognized in cancer research and in the study of embryonic development in

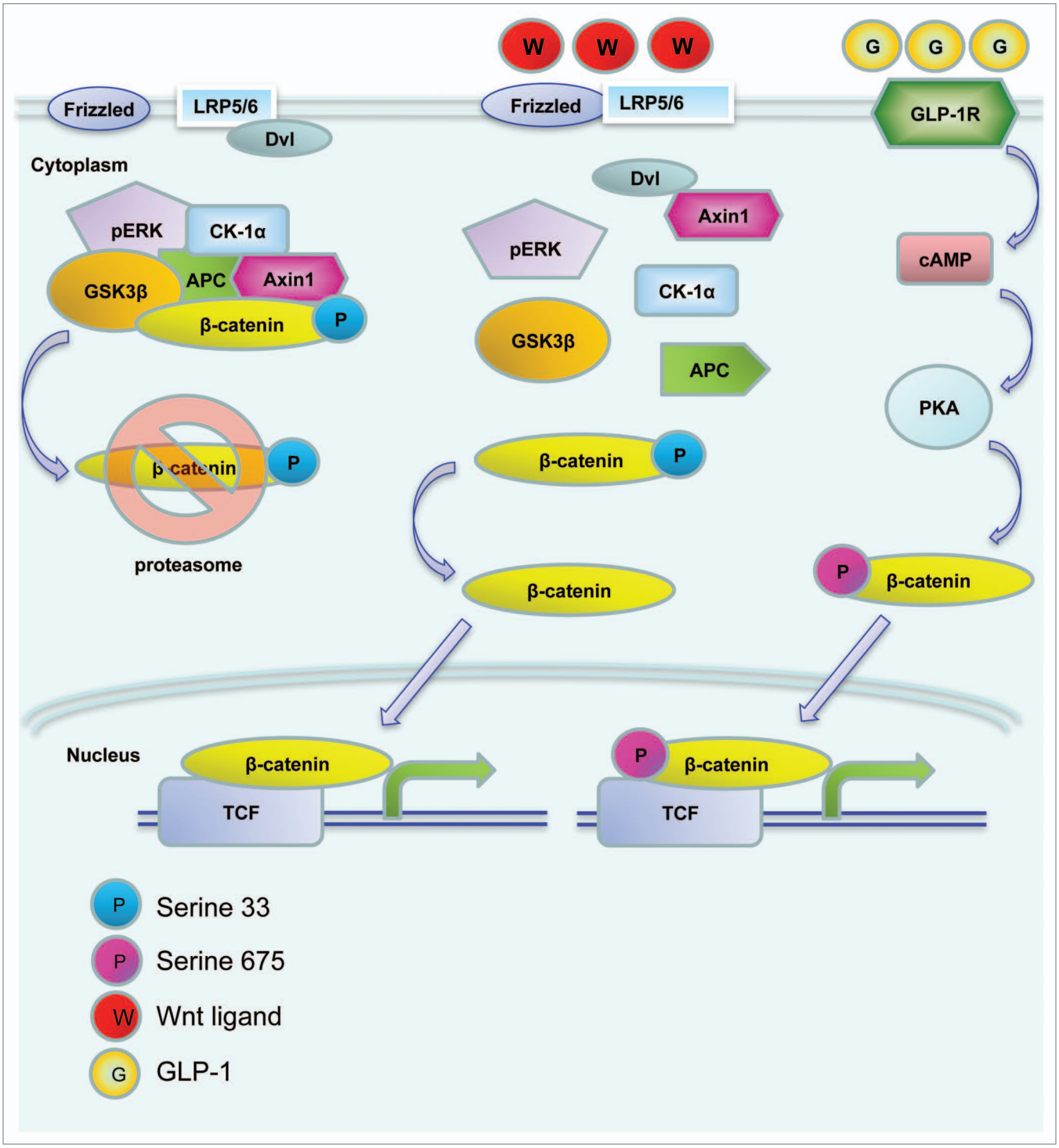

Figure 2. An illustration of the Wnt signaling pathway. Without Wnt ligand stimulation, β-cat is trapped within the "destruction complex," phosphorylated by the protein kinase GSK-3 and CK-1α at Ser33 and adjacent Ser positions, and subsequently degraded by proteasome (left). Following Wnt ligand stimulation and Dishvelled (Dvl) activation, β-cat escapes the trapping, enters the nucleus and forms the bipartite transcription factor cat/TCF, which leads to the stimulation of Wnt target gene expression (middle). GLP-1 was shown to activate cAMP-dependent protein kinase A (PKA), and stimulate β-cat Ser675 phosphorylation, which is positively associated with its nuclear translocation and Wnt target gene expression (right).

Xenopus and *Drosophila*. The involvement of this signaling cascade in organogenesis in mammals has also been extensively investigated. Wnt ligands, through their cell membrane bound Frizzled receptors and the LRP5/6 co-receptor, exert various fundamental physiological and pathophysiological functions in mammals and other species. As shown in **Figure 2**, the major downstream effector of the canonical Wnt signaling pathway (defined as Wnt pathway hereafter) is β-cat/TCF or cat/TCF. This bipartite

transcription factor consists of free β-cat and a member of the TCF family (TCF7, LEF-1, TCF7L1 and TCF7L2). The level of cytosolic free β-cat is tightly controlled by the proteasome-mediated degradation process, involving the tumor suppressors adenomatous polyposis coli (APC) and axin, as well as the serine/threonine kinases glycogen synthase kinase-3 (GSK-3) and casein kinase 1α (CK1α). β-cat is phosphorylated by GSK-3 and CK1α at Ser33 and three other adjacent serine positions to facilitate its proteasome degradation. Wnt ligands exert their regulatory effects via a Frizzled receptor and the LRP5/6 co-receptor. Upon Wnt ligand binding, the Wnt receptor associates with the protein known as dishevelled (Dvl). This event triggers the disruption of the complex containing APC, axin, GSK-3 and β-cat, preventing it from phosphorylation-mediated degradation. β-cat will be able to enter the nucleus and form the β-cat/TCF bipartite transcription factor, leading to elevated expression of target genes that are downstream of Wnt (or cat/TCF) signaling cascade.

Extensive recent studies have revealed that cat/TCF also mediates the effect of signaling molecules other than the Wnt ligands.[47] A battery of peptide hormones, including GLP-1, that utilize cAMP as their second messenger, have been shown to exert their functions through the Wnt effector β-cat/TCF.[47] This has been attributed to PKA mediated activation of β-cat phosphorylation at a C-terminal Ser675 or Ser552 position (**Fig. 2**).

β-cat and GLP-1 production. We demonstrated previously that both lithium (which mimics the function of the Wnt ligands)[48] and a constitutively active β-cat molecule (carrying S33Y mutation) can stimulate the activity of the rat *gcg* promoter.[49] Lithium was also shown to stimulate endogenous *gcg* mRNA expression and GLP-1 production in mouse intestinal *gcg*-expressing cell lines and in primary fetal rat intestinal cell cultures.[49] More recently, we found that *gcg* promoter and mRNA expression can be stimulated by Wnt-3A.[50] Activation of *gcg* promoter by lithium treatment is dependent upon a TCF binding motif located within the G2 element of the *gcg* promoter.[51] As this G2 enhancer element is known to mediate the stimulatory effects of cAMP and calcium,[52] this observation suggested that cAMP activates *gcg* expression via cross-talking with the Wnt signaling pathway effector cat/TCF. Using chromatin immunoprecipitation (ChIP), we have then demonstrated an in vivo physical interaction between TCF7L2 and the G2 element.[51] Interestingly, GIP expression was also found to be stimulated by the Wnt signaling cascade by García-Martínez and colleagues.[53]

β-cat and GLP-1 function. cat/TCF can mediate the function of many hormonal and other regulatory peptides during the adulthood.[54] This can be achieved by β-cat Ser675 phosphorylation by cAMP/PKA or insulin/PAK-1 signaling cascade.[50,55-57] Liu and Habener have assessed the involvement of TCF7L2, β-cat and Wnt signaling pathway in the function of GLP-1 and the GLP-1R agonist exendin-4.[12] They demonstrated the expression of TCF7L2 in the rat INS-1 cell line and determined the presence of Wnt activity in mouse pancreatic islets using the TOPGAL transgenic mouse model. In this mouse, the expression of β-galactosidase reporter is controlled by a regulatory DNA element consisting of consensus LEF/TCF binding site upstream of a minimal c-*fos* promoter.[58] Liu and Habener found that islets from TOPGAL mice show increased LacZ expression in response to exendin-4 treatment.[12] This, along with a battery of other observations obtained by Liu and Habener, suggests that β-cat/TCF, the effector of the Wnt signaling, mediates the function of GLP-1 in stimulating β-cell proliferation. They have also demonstrated the activation of β-cat Ser675 phosphorylation by exendin-4.[12] We found very recently that GLP-1(28–36) can also stimulate β-cat Ser675 phosphorylation, along with the stimulation of cAMP/PKA signaling cascade (unpublished data of Shao et al.).

Summary and Perspective

Although the studies on the two incretin hormones during the past two decades has led to the development of GLP-1R agonists and DPP-IV inhibitors as therapeutic agents for diabetes and possibly other metabolic disorders, further exploration of mechanisms underlying the function of GLP-1 will be helpful not only for the identification of novel drug targets, but also for the improvement of the usage of existing drugs.

GLP-1 is different from another incretin hormone GIP, as it possesses the insulinomimetic effect in pancreatic β-cells and extra-pancreatic tissues. The pancreatic insulinomimetic effect of GLP-1 makes its analogs or GLP-1R agonists more attractive agents in the treatment of not only type 2 diabetes mellitus, but also type 1 diabetes mellitus. The exploration of mechanisms underlying the extra-pancreatic insulinomimetic effect of GLP-1, including that in heart, liver, muscle, brain and elsewhere, may lead to the identification of novel therapeutic targets for type 2 diabetes mellitus, obesity, and other metabolic disorder.

It becomes clear to us that previously assumed "degradation" products of GLP-1(7–36) possess, at least, potential pharmacological functions. Although the degradation process may serve as a negative feedback for attenuating its acute insulinotropic effect, the products generated may exert their insulinomimetic effects on various organs. In the next few years, we anticipate to see the studies in elucidating whether these effects are mediated by a novel receptor or through a receptor-independent mechanism.

Basic research on the Wnt signaling pathway in different disciplines have revealed that the bipartite transcription factor cat/TCF mediates not only the effect of the Wnt ligands, but also the cell signaling of certain peptide hormones, especially those that utilize cAMP as a second messenger.[47] Evidently, β-cat posttranslational modification, especially its C-terminal phosphorylation will be the focus for further functional study of GLP-1. As shown in **Figure 3**, GLP-1(7–36)amide is capable of stimulating β-cat Ser 675 phosphorylation, leading to Wnt signaling pathway activation. Since the GLP-1R agonist exendin-4 can also stimulate β-cat Ser-675 phosphorylation, we anticipate that this event can be mediated by GLP-1R(12). GLP-1(9–36) amide generated by DPP-IV cleavage exerts certain beneficial effects in a GLP-1R independent manner.[5] The further cleavage by NEP 24.11 leads to the generation of GLP-1(28–36)amide. This peptide is known to possess the cytoprotective effect for

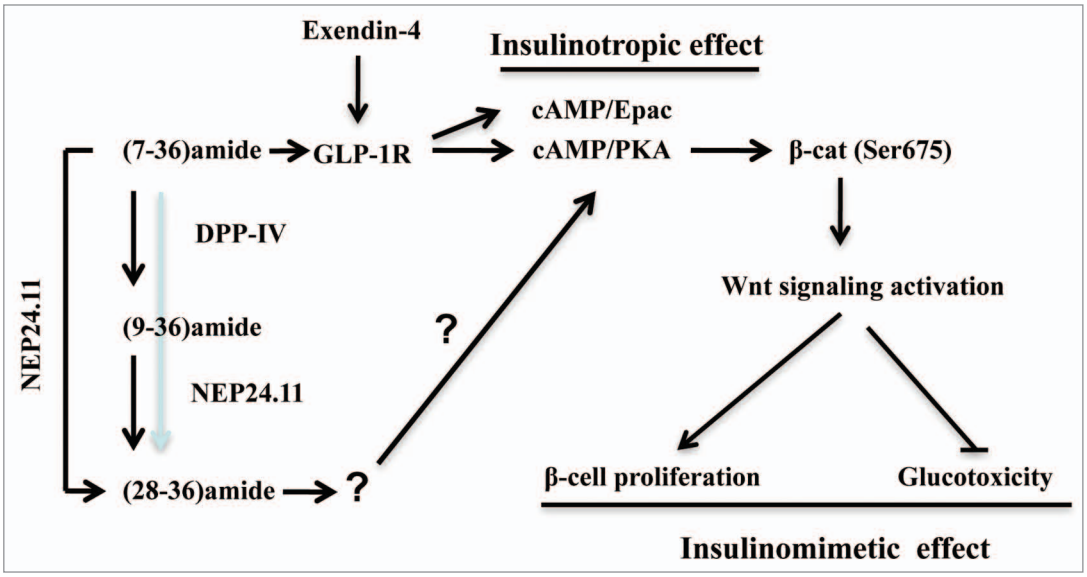

Figure 3. Summary of insulinotropic and insulinomimetic effects of GLP-1 and its cleavage products in pancreatic β-cells. The cleavage of GLP-1(7–36) amide (defined as 7–36amide) by DPP-IV leads to the production of GLP-1(9–36)amide (defined as 9–36amide). The cleavage by NEP 24.11 leads to the production of GLP-1 (28–36)amide (defined as 28–36amide). GLP-1R mediates the insulinotropic effect of 7–36amide and GLP-1R agonists, such as exendin-4, involving both cAMP/PKA and cAMP/Epac. PKA can activate β-cat via increasing its Ser675 phosphorylation, which is at least partially responsible for the insulinomimetic effect of GLP-1. Whether 28–36amide exerts its insulinomimetic effect in pancreatic β-cells via a yet to be identified receptor, or a receptor independent mechanism remain to be further investigated. Whether or not 28–36amide exerts its insulinomimetic effect via stimulating β-cat Ser675 phosphorylation is also worth to be further examined.

pancreatic β-cells in vitro, and inhibits weight gain and attenuates diabetes and hepatic steatosis in high fat diet-induced obese mouse model.[8,9,25] Whether this nonapeptide activates β-cat and functions as a powerful insulinomimetic hormone for both pancreatic β-cells and extra-pancreatic organs are worth to be examined.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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