GPR40: A therapeutic target for mediating insulin secretion (Review)

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Abstract. G-protein-coupled receptor 40 (GPR40), known as free fatty acid receptor 1, is mainly expressed in pancreatic β-cells and activated by medium- and long-chain fatty acids. Increasing evidence indicates that the activation of GPR40 in cells causes insulin secretion, and GPR40 has become an attractive therapeutic target for type 2 diabetes. Recently, certain novel GPR40 agonists have been identified that regulate glucose-stimulated insulin secretion, leading to the development of new drugs for the treatment of type 2 diabetes. In this review, we focus on progress in the physiological role of GPR40 and potential drugs targeting GPR40 over the past decade.

Contents

1. Introduction

Type 2 diabetes is one of the crucial health problems worldwide, and its prevalence is rising dramatically (1,2). In addition to insulin resistance, pancreatic β-cell dysfunction typically characterized by progressive decreases in glucose-stimulated insulin secretion (GSIS), is another hallmark of type 2 diabetes. As is well known, plasma glucose is a leading adaptor in mediating insulin secretion. Elevated blood glucose levels cause glucose to diffuse into β-cells through the non-insulin-dependent glucose transporter-2 (Glut2) and then glucose metabolism results in the production of adenosine triphosphate (ATP). Increased ATP levels, particularly a rise in the ratio of ATP/adenosine diphosphate (ADP), lead to the closure of K^+-ATP channels and subsequent plasma membrane depolarization. This opens voltage-dependent Ca^{2+} channels and causes subsequent Ca^{2+} influx, and increased cytoplasmic free Ca^{2+} levels prompt insulin granule exocytosis, thus triggering insulin secretion (3) (Fig. 1).

Additionally, free fatty acids (FFAs) are not only essential dietary nutrients, but play a crucial role in the modulation of insulin secretion (4-6). It is believed that prolonged exposure to elevated FFAs results in impaired insulin secretion involving lipotoxicity which exerts the deleterious effects of lipid accumulation on β-cell secretory function by inhibiting insulin biosynthesis (7,8), promoting programmed cell death (9) and inducing reactive oxidant species (ROS) generation and inflammatory reaction (10,11). However, recent evidence indicates that not all FFAs inhibit insulin secretion. The long-term *in vitro* treatment of INS-1 rat pancreatic β-cells by polyunsaturated α-linolenic acid does not reduce insulin secretion and saturated palmitic acid-induced suppression of basal insulin secretion and GSIS is attenuated by α-linolenic acid (12). On the contrary, FFAs acutely enhance basal insulin secretion and GSIS, and the molecular mechanisms involve the surface receptors of pancreatic β-cells. Over the past decades, a series of receptors have been identified for FFAs, such as the nuclear receptors, peroxisome proliferator-activated receptors (PPARs), fatty acid-binding proteins (FABPs) and G-protein-coupled receptors (GPRs), a large family of cell surface receptors (13,14). Moreover, cell surface receptors have been proven to play a key role in FFA biological processes, which suggests that FFAs do not need to enter into the cells to elicit their effects. Recently, GPRs have been successfully identified as multiple cell surface receptors for FFAs, also known as FFA receptors (FFARs). Among these, GPR41 and GPR43, known as FFAR3 and FFAR2, respectively, are

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activated by short-chain fatty acids, such as formate, acetate, propionate and butyrate (15), while GPR40 and GPR120 by medium- and long-chain ones, such as palmitate, palmitoleate and oleate (16,17). GPR41 is expressed in adipose tissue and the gastrointestinal tract, and short-chain FFAs induce leptin secretion from adipocytes by stimulating GPR41, suggesting that GPR41 regulates energy homeostasis (18). GPR43 has been detected in immune cells, adipocytes and the gastrointestinal tract, and contributes to inflammatory responses and metabolic homeostasis (19-21). GPR120, primarily expressed in the intestine and macrophages, promotes glucagon-like peptide-1 (GLP-1) secretion from the intestine and represses macrophage-induced inflammation (22,23). GPR40, namely FFAR1, is executively expressed in pancreatic β-cells and mediates insulin secretion upon medium- and long-chain FFA stimulation (16,24). Type 2 diabetes islets have a lower GPR40 expression with impaired insulin secretion (25) and GPR40 knockout leads to decreases in both glucose- and arginine-stimulated insulin secretion *in vivo* without changes in insulin sensitivity (26). The overexpression of GPR40 in pancreatic β-cells augments GSIS and improves glucose tolerance in normal and diabetic mice (27), and the GPR40 agonist also displays the same effects in rodents (28). Therefore, GPR40 has received considerable attention as a potential therapeutic target for type 2 diabetes, and a series of novel agonists for GPR40 have been found, leading to the development of new drugs for type 2 diabetes (29,30).

2. Gene and protein information for GPR40

In addition to being expressed in pancreatic β-cells in a range of species including mice, rats and humans (6,31,32), GPR40 is expressed at very low levels in all other tissues (16,33). The GPR40 gene is located on human chromosome 19q13.1, and shares approximately 30-40% identity with GPR41 and GPR43 (14). The mouse gene is composed of a 24-bp non-coding exon, a 698-bp intron and a 4402-bp second exon, of which the intron is located between the 2 exons and is spliced out during RNA processing, and the second exon contains the full coding sequence. Three evolutionarily conserved sequences (HR1-HR3) are located upstream of the first exon. Among these, HR2 is a potent β-cell-specific enhancer and binds transcription factors, PDX1 and BETA2, and is thus responsible for regulating the transcriptional levels of the gene in β-cells (34,35). GPR40 belongs to class A GPRs, showing a typical 7-transmembrane (TM) domain structure spanning α-helices with 3 hydrophilic intracellular and 3 hydrophilic extracellular loops. The N-terminus is located extracellularly while the C-terminus resides in the cytosol (36). Although the full protein information for GPR40 has not yet been revealed, its structure has been analyzed by computational modeling, site-directed mutagenesis and so on. GPR40 contains 2 sites (Thr215 and Ser298) bearing potential protein kinase C (PKC) phosphorylation and 2 putative N-glycosylation ones (Asn155 and Asn165) (37). Additionally, the anchored sites of fatty acids are determined in amino residues Arg183, Asn244 and Arg258 located close to the extracellular domains of TM5, 6 and 7 when GPR40 is stimulated. In the resting state, the Arg183 and Arg258 residues consist of an ionic lock with 2 glutamate residues (Glu145 and Glu172) located in TM2. In the presence of ligands, however, the ionic lock is broken, and then Arg183 and Arg258 are anchored by fatty ligands (37-40). A recent study revealed that His137 directly refers to ligand recognition through the NH-π interaction with GW9508, while His86 does not interact with GW9508 in the NH-π interaction (41). It has been proven that the Arg211His and Gly180Ser polymorphisms in the GPR40 gene are strongly linked to receptor functionality and insulin secretion (42,43).

3. Ligands

Three independent groups have reported that GPR40 is activated by medium- and long-chain FFAs in the micromolar concentration range (44-46), including saturated fatty acids and unsaturated fatty acids. The agonistic activity of the former is dependent on chain length from at least 10 carbon atoms (capric acid) to as many as 23 (tricosanoic acid), of which pentadecanoic acid (C15) and palmitic acid (C16) display the most potent agonistic activity, while capric acid (C10) demonstrates either weak or no activity. However, the latter, including a variety of monounsaturated fatty acids such as 9Z-palmitoleic acid (C16:1) and 9Z-oleic acid (C18:1) and polyunsaturated fatty acids such as 9Z,12Z-linoleic acid (C18:2) and 5,8,11-eicosatrienoic acid (C20:3), do not appear to be dependent on carbon chain length or the degree of saturation (44). Additionally, a series of synthetic agonists such as GW9508, AMG 837 and 3-substituted 3-(4-aryloxyaryl)-propanoic acids have been recently reported (47-50). Several synthetic GPR40 antagonists have also been well described, including DC260126 and GW1100 (51-54).

4. Physiological role of GPR40 in mediating insulin secretion

Elevated plasma FFAs often co-exist with type 2 diabetes and obesity, and fatty acids play an important role in insulin secretory function of β-cells. In the absence of fatty acids, GSIS is impaired. On the contrary, an increase in blood FFA concentration augments GSIS (55). Contrary to the acute effects of FFAs, chronically elevated fatty acids have been strongly linked to reduced insulin secretion (56). However, it is not always true that all FFAs impair β-cell function; prolonged exposure to unsaturated FFAs, such as polyunsaturated α-linolenic acid but not saturated palmitic acid protects β-cell function and augments GSIS (12). As a receptor of medium- and long-chain FFAs, GPR40 has been well documented to contribute to insulin secretion. GPR40 is highly expressed in human pancreatic β-cells, islet cell tumors and various pancreatic-derived cell lines, including INS-1E, MIN6, β-TC-3 and HIT-T15. Islets from type 2 diabetic patients chronically exposed to FFA have a lower GPR40 mRNA expression than those from non-diabetic multiorgan donors, following impaired insulin secretion (25,57). Likewise, the deletion of GPR40 decreases GSIS *in vivo* in mice without affecting intracellular fuel metabolism in islets and insulin sensitivity (26,58), and the islets from GPR40 knockout mice have a reduced capacity to release insulin in response to fatty acids (26,59), which suggests that GPR40 is required for normal insulin secretion. The overexpression of GPR40, however, improves glucose tolerance with an increase in insulin secretion in normal and diabetic mice. Moreover,

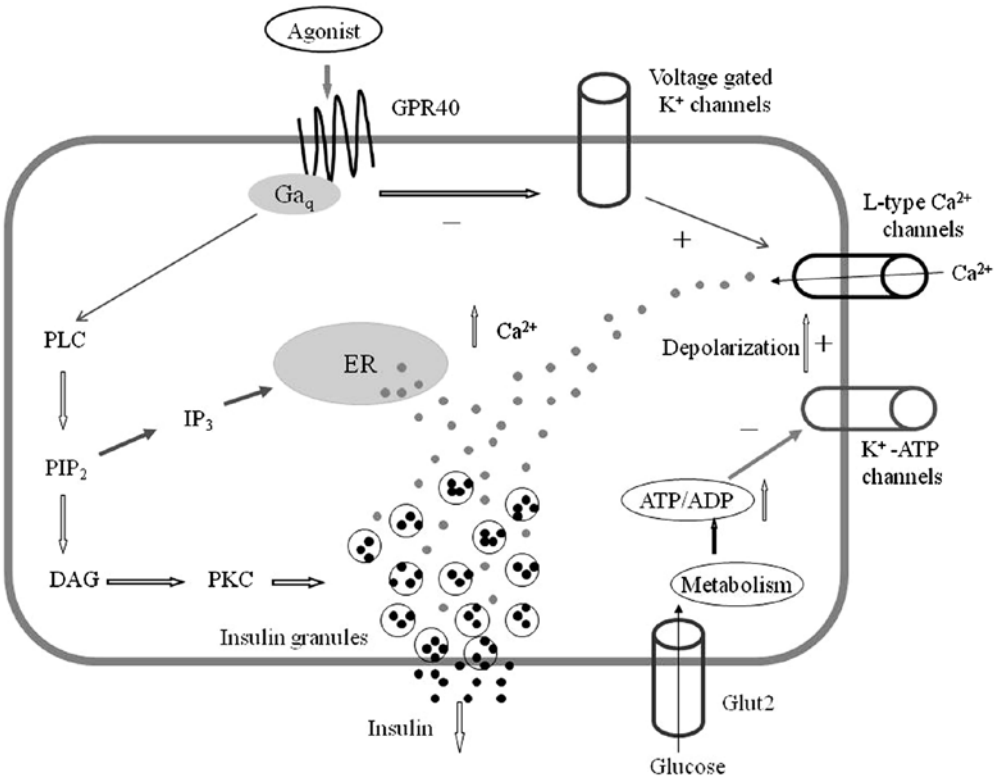

Figure 1. GPR40 signaling in pancreatic β-cells.

mice have been found to be resistant to high-fat diet-induced glucose intolerance, and isolated islets from mice potentiate enhanced insulin secretion in response to high glucose and resist the impairment of β-cells in insulin secretion against prolonged palmitate exposure (27).

Upon stimulation by agonists, GPR40 couples to the Ca^{2+}-mobilizing G protein, Ga_q (44). This results in phospholipase C (PLC) activation which promotes plasma membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Then, IP_3 transfers into the endoplasmic reticulum (ER), thus leading to the release of stored Ca^{2+} from ER and subsequently increased cytoplasmic Ca^{2+} levels, while DAG potentiates insulin secretion by stimulating PKC (9,16). GPR40 knockout contributes to the decrease in lyso-phosphatidylethanolamine species and absence in intracellular inositol phosphate levels in response to fatty acids and subsequent reduction in GSIS (26). The inhibition of PLC or L-type Ca^{2+} channels attenuates rises in GPR40-dependent Ca^{2+} levels and insulin secretion stimulated by fatty acids; the same effects are observed in GPR40 knockout β-cells (60-62). This process suggests that GPR40 mediates insulin secretion involving Ca^{2+} release from ER and influx via L-type Ca^{2+} channels. Moreover, linoleic acid reversibly reduces the voltage-gated K^+ current in rat β-cells through GPR40, while GPR40-specific small interfering RNA significantly reduce the decrease in K^+ current induced by linoleic acid. Taken together, these data indicate that activated GPR40 inhibits the opening of voltage-gated K^+ channels, resulting in increased Ca^{2+} influx via L-type Ca^{2+} channels, and therefore enhancing the depolarization of the plasma membrane, thereby augmenting GSIS (63) (Fig. 1).

5. Emerging potential drugs targeting GPR40 for the regulation of insulin secretion

Due to tissue distribution, the pharmacological activation of GPR40 provides a novel target for the treatment of type 2 diabetes. Certain synthetic GPR40 agonists are very promising to become the drug for mediating insulin secretion. For example, GW9508, a small molecule agonist, activates GPR40 and stimulates GSIS in MIN6 cells, implicating a potential glucose-sensitive insulin secretagogue (64). A phenylpropanoic acid derivative named 3-{2-fluoro-4-[({4'-[(4-hydroxy-1,1-dioxidotetrahydro-2H-thiopyran-4-yl) methoxy]-2',6'-dimethylbiphenyl-3-yl}methyl)amino]phenyl} propanoic acid has been shown to exhibit a robust plasma glucose-lowering effect and insulinotropic action during an oral glucose tolerance test in rats with impaired glucose tolerance (65). AMG 837 is a potent GPR40 agonist with a superior pharmacokinetic profile, improving glucose intolerance and promoting GSIS in rodents (28,66). Although thiazolidinediones including pioglitazone, the insulin sensitizers, are proven to activate GPR40 and reverse palmitate-induced β-cell dysfunction (67,68), the adverse effects such as heart problems and bone fractures have already been reported (69,70). Recently, Zhou *et al* (71) discovered a series of thiazolidinediones (TZDs) as potent GPR40 agonists by systematic structure-activity relationship studies of a screening. Among these, compound C demonstrated an acute mechanism-based glucose lowering in an intraperitoneal glucose tolerance test (IPGTT) in lean mice, while no effects were observed in GPR40 knockout mice. However, it is necessary to determine whether compound C has the same adverse effects.

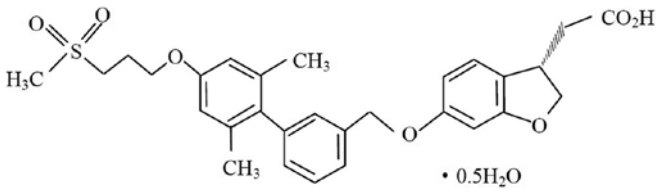

Figure 2. Chemical structure of TAK-875 (74).

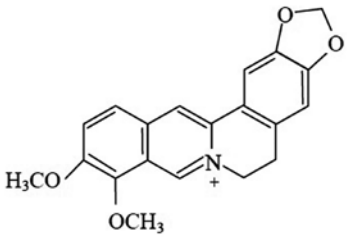

Figure 3. Chemical structure of berberine.

TAK-875, a GPR40-selective agonist (Fig. 2), enhances insulin secretion in a glucose-dependent manner in both isolated rat and human islets, which correlates with the elevation of intracellular inositol monophosphate and Ca^{2+} concentration, similar to that produced by GLP-1 (72,73), thus minimizing the risk of hypoglycemia and representing a therapeutically useful feature. The oral administration of TAK-875 significantly improves both fasting hyperglycemia and glucose tolerance and augments GSIS in type 2 diabetic rats with no evidence of β-cell toxicity, showing promising pharmacokinetic profiles (73,74). Further research has revealed that TAK-875 is well tolerated in healthy volunteers and has pharmacokinetic characteristics suitable for a once daily regimen, and pharmacodynamic data have shown that TAK-875 has a low risk of hypoglycemia (75). At present, the agonist has been studied in clinical trials (76,77), including a phase II, multicentre, randomized, double-blind, parallel group study. After treatment, TAK-875 led to reductions in blood glucose levels and HbA1c and an increase in insulin levels. Moreover, no episode of hypoglycemia was observed despite the significant reduction in plasma glucose levels. These findings indicate that the GPR40 agonist, TAK-875, is a glucose-dependent insulinotropic reagent and a promising clinical drug for the treatment of type 2 diabetes.

In China, a number of Chinese herbs have been shown to possess antidiabetic activities with few adverse effects (78), including *Coptis chinensis*, *Astragalus membranaceus* and *Lonicera japonica* (79,80); however, the mechanisms involved remain unclear. Berberine, a botanical alkaloid (Fig. 3) extracted from *Coptis chinensis* Franch., has been used to treat type 2 diabetes in clinical practice. Although certain gastrointestinal complaints from berberine treatment, including slight constipation appear to be associated with the use of high doses, the tolerability is high for low doses (81). Berberine performs a series of pharmacological functions, including anti-inflammation, ameliorating insulin resistance and has a protective effect on β-cell lipoapoptosis (82,83). It is not only an insulin sensitizer via enhancing glucose metabolism in insulin-sensitive tissues, but also an insulinotropic reagent. Previous studies have revealed that berberine stimulates glucose-dependent insulin secretion from rat pancreatic β-cells and exhibits a dose-dependent increase in calcium mobilization in a GPR40-overexpressed cell line, similar to oleic acid, a GPR40 agonist (84,85). Therefore, it is possible that berberine is a novel agonist of GPR40. Additionally, *Rehmanniae radix*, *Ginseng radix* and *Scutellariae radix* have also been shown to have the potential to improve GSIS (86,87).

6. Conclusion and perspectives

GPR40 is no doubt a novel therapeutic target for type 2 diabetes involving mediating insulin secretion, and a series of agonists for GPR40 have developed. However, further studies are warranted to determined the safety, tolerability, pharmacokinetic and pharmacodynamic properties. At present, a promising clinical drug targeting GPR40 is TAK-875, which is currently undergoing phase II, multicentre, randomized, double-blind, placebo-controlled trials. However, adverse effects including long-term reaction are not yet clear. GPR40 is also expressed in enteroendocrine and glial cells, and the ligands display toxic activity (88-90). Therefore, it is necessary to confirm whether TAK-875 affects these tissues. Additionally, Chinese medicine has been used to treat diabetes for thousands of years in clinical practice with few side-effects, and has become popular complementary and alternative medicine. A number of Chinese herbs have been proven to protect pancreatic β-cell function and to regulate insulin secretion. It is worthwhile to screen insulinotropic reagents from Chinese herbs and to develop GPR40 agonists. This may lead to a reduction in research funds and time.

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