Overexpression of GPR40 in Pancreatic β-Cells Augments Glucose-Stimulated Insulin Secretion and Improves Glucose Tolerance in Normal and Diabetic Mice

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OBJECTIVE—GPR40 is a G protein–coupled receptor regulating free fatty acid–induced insulin secretion. We generated transgenic mice overexpressing the hGPR40 gene under control of the mouse insulin II promoter and used them to examine the role of GPR40 in the regulation of insulin secretion and glucose homeostasis.

RESEARCH DESIGN AND METHODS—Normal (C57Bl/6J) and diabetic (KK) mice overexpressing the hGPR40 gene under control of the insulin II promoter were generated, and their glucose metabolism and islet function were analyzed.

RESULTS—In comparison with nontransgenic littermates, hGPR40 transgenic mice exhibited improved oral glucose tolerance with an increase in release. Although islet histology showed no obvious differences between hGPR40 transgenic and nontransgenic mice, isolated islets from hGPR40 transgenic mice had enhanced insulin secretion in response to high glucose (16 mmol/l) compared with those from nontransgenic mice, and they both had similar low glucose (3 mmol/l)-stimulated insulin secretion. In addition, hGPR40 transgenic islets significantly increased insulin secretion in a naturally occurring agonist palmitate in the presence of 11 mmol/l glucose. hGPR40 transgenic mice were also found to be resistant to high-fat diet–induced glucose intolerance, and hGPR40 transgenic mice harboring KK background showed augmented insulin secretion and improved oral glucose tolerance compared with nontransgenic littermates.

CONCLUSIONS—Our results suggest that GPR40 may have a role in regulating glucose-stimulated insulin secretion and plasma glucose levels in vivo and that pharmacological activation of GPR40 may provide a novel insulin secretagogue beneficial for the treatment of type 2 diabetes. Diabetes 58:1067–1076, 2009

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insulin secretion both in regular and high-fat–diet feeding conditions. Moreover, even when insulin resistance was reinforced in diabetic KK mice, overexpression of hGPR40 in this background also improved glucose tolerance with increasing insulin secretion. Thus, our findings indicated that GPR40 has a role in regulating glucose-stimulated insulin secretion and plasma glucose levels in vivo, and they supported the concept that GPR40 agonists might be effective insulin secretagogues for the treatment of type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Generation of hGPR40 transgenic mice.** The transgene consisted of 0.7 kb of mouse insulin II gene promoter, followed by 2.2 kb of the hGPR40 gene, including the 3′ noncoding region (8), and the complete 2.9-kb fragment was purified and microinjected into the fertilized eggs of C57BL/6J mice (CLEA Japan, Tokyo) (23). The transgenic mice obtained were maintained by crossing with C57BL/6J mice, and the transgenic founder mice were identified by PCR analyses of tail DNA using the hGPR40 gene–specific primers 5′-GGAGTGTGTTGCTCCTAATCCGGTGT-3′ and 5′-AGACGTGCTCTCCTCTTGTAAGTACAA-3′. When examining the hGPR40 transgenic mice harboring KK hybrid background, hGPR40 transgenic mice were crossed with mice harboring a KK background, and the mice obtained contained almost 50% KK background (hGPR40 transgenic × KK). The transgenic mice were identified by PCR analyses of tail DNA using the hGPR40-specific primers described above. Age- and sex-matched littermates were used as control mice throughout the study, and all experiments were performed using male mice, unless otherwise stated. The mice were fed with a regular diet containing 11.5 kcal% fat (CE-2; CLEA Japan) and were housed in colony cages and maintained on a 12-h light/dark cycle with free access to food and water. When examining the effects of high-fat–diet feeding, mice were fed with a high-fat diet containing 60 kcal% fat (D12492; Research Diets, New Brunswick, NJ) from 8 weeks of age. The genotype of mice was confirmed using an avidin-biotin detection system (Ventana Medical Systems, Tucson, AZ) and an M.O.M. immunodetection kit (Vector Laboratories, Burlingame, CA), according to the manufacturer’s instructions. Antigen retrieval was performed using citrate buffer (pH 6.0) for 30 min at 110°C. After antigen retrieval, the sections were blocked with 3% H2O2 for 30 min and then incubated with primary antibodies. When examining the hGPR40 transgenic mice (CLEA Japan, Tokyo), the transgenic mice obtained were main- 0.7 kb of the hGPR40 gene, including the 3′ noncoding region (8), and the complete 2.9-kb fragment was purified and microinjected into the fertilized eggs of C57BL/6J mice (CLEA Japan, Tokyo) (23). The transgenic mice obtained were maintained by crossing with C57BL/6J mice, and the transgenic founder mice were identified by PCR analyses of tail DNA using the hGPR40 gene–specific primers 5′-GGAGTGTGTTGCTCCTAATCCGGTGT-3′ and 5′-AGACGTGCTCTCCTCTTGTAAGTACAA-3′. When examining the hGPR40 transgenic mice harboring KK hybrid background, hGPR40 transgenic mice were crossed with mice harboring a KK background, and the mice obtained contained almost 50% KK background (hGPR40 transgenic × KK). The transgenic mice were identified by PCR analyses of tail DNA using the hGPR40-specific primers described above. Age- and sex-matched littermates were used as control mice throughout the study, and all experiments were performed using male mice, unless otherwise stated. The mice were fed with a regular diet containing 11.5 kcal% fat (CE-2; CLEA Japan) and were housed in colony cages and maintained on a 12-h light/dark cycle with free access to food and water. When examining the effects of high-fat–diet feeding, mice were fed with a high-fat diet containing 60 kcal% fat (D12492; Research Diets, New Brunswick, NJ) from 8 weeks of age. The genotype of mice was confirmed using an avidin-biotin detection system (Ventana Medical Systems, Tucson, AZ) and an M.O.M. immunodetection kit (Vector Laboratories, Burlingame, CA), according to the manufacturer’s instructions. Antigen retrieval was performed using citrate buffer (pH 6.0) for 30 min at 110°C. After antigen retrieval, the sections were blocked with 3% H2O2 for 30 min and then incubated with primary antibodies. When examining the hGPR40 transgenic mice (CLEA Japan, Tokyo), the transgenic mice obtained were main-
was significantly lower than that in nontransgenic mouse (Fig. 2A, B, and E). In parallel with improvement of glucose tolerance, insulin responses to glucose at the early phase were higher in both hGPR40 transgenic lines than in nontransgenic mice (Fig. 2C and D), and AUC_{0-30 min} of plasma insulin was significantly increased in both lines of hGPR40 transgenic mice compared with nontransgenic mice (Fig. 2F). Whole-body insulin sensitivity was assessed by the insulin tolerance test, and no apparent differences were observed between hGPR40 transgenic and nontransgenic mice (Fig. 2G and H).

**Islet structure and β-cell function in vitro.** We next examined whether hGPR40 overexpression affects islet structure. At 16 weeks of age, immunohistochemical analysis of pancreas sections with antibodies against insulin, glucagon, GLUT2, and proinsulin showed no apparent differences between hGPR40 transgenic (47M and 23F) and nontransgenic mice (Fig. 3A). β-Cell areas of 47M and 23F lines were almost the same as those of nontransgenic mice (Fig. 3B). To further examine the effects of the hGPR40 overexpression on islet function, isolated islets from hGPR40 transgenic (47M and 23F) mice and nontransgenic mice at 9 weeks of age were stimulated with glucose or glucose plus palmitate in vitro, and secreted insulin was measured. Basal insulin secretion at low glucose concentration (3 mmol/l) was similar between hGPR40 transgenic and nontransgenic islets, but isolated islets from hGPR40 transgenic mice had enhanced insulin secretion in response to high glucose (16 mmol/l) compared with islets from nontransgenic mice (Fig. 3C). Similar results were obtained when using islets isolated from the 23F line (Fig. 3D). Stimulation with palmitate, one of the naturally occurring agonists of GPR40, significantly increased insulin secretion in islets of hGPR40 transgenic mice compared with those of nontransgenic mice at 11 mmol/l glucose, indicating that the expressed hGPR40 might be functional in β-cells of hGPR40 transgenic mice (Fig. 3E). These results suggest that the quantity of insulin secretion was more enhanced than that of insulin synthesis in hGPR40 transgenic mice islets.

**Effects of high-fat–diet feeding on glucose homeostasis in hGPR40 transgenic mice.** Because GPR40 is the receptor for medium- and long-chain FFAs (8–10), and a

| TABLE 1 |
|---|
| Metabolic parameters in hGPR40 transgenic (47M and 23F) mice fed a regular diet or a high-fat diet |
| **Body weight (g)** | **Plasma glucose (mg/dl)** | **Plasma insulin (ng/ml)** | **Plasma NEFA (mEq/l)** |
|---|---|---|---|
| **Regular diet** | | | |
| Fed | Nontransgenic | 28.8 ± 0.4 | 170.0 ± 4.1 | 1.54 ± 0.30 | 0.33 ± 0.08 |
| | Transgenic (47M) | 27.5 ± 0.6 | 153.6 ± 6.2 | 1.61 ± 0.43 | 0.24 ± 0.06 |
| | Nontransgenic | 28.7 ± 0.4 | 187.6 ± 9.8 | 1.43 ± 0.38 | 0.37 ± 0.08 |
| | Transgenic (23F) | 28.1 ± 0.3 | 161.4 ± 8.1 | 1.46 ± 0.38 | 0.41 ± 0.06 |
| Fasted | Nontransgenic | 24.3 ± 0.4 | 106.0 ± 4.8 | 0.19 ± 0.04 | 1.35 ± 0.06 |
| | Transgenic (47M) | 22.9 ± 0.6 | 84.5 ± 4.4* | 0.23 ± 0.05 | 1.00 ± 0.07 |
| | Nontransgenic | 24.0 ± 0.3 | 117.7 ± 5.0 | 0.21 ± 0.03 | 1.07 ± 0.07 |
| | Transgenic (23F) | 23.6 ± 0.3 | 89.9 ± 5.2* | 0.24 ± 0.05 | 0.98 ± 0.07 |
| **High-fat diet** | | | |
| Fed | Nontransgenic | 49.9 ± 0.7 | 215.6 ± 8.1 | 18.87 ± 3.52 | 0.52 ± 0.04 |
| | Transgenic (47M) | 49.6 ± 1.0 | 212.8 ± 9.9 | 16.17 ± 2.95 | 0.49 ± 0.03 |
| Fasted | Nontransgenic | 46.0 ± 0.6 | 173.8 ± 5.6 | 2.88 ± 0.31 | 0.80 ± 0.04 |
| | Transgenic (47M) | 45.4 ± 0.9 | 133.3 ± 4.5* | 3.07 ± 0.39 | 0.88 ± 0.04 |

All parameters were measured in the fed and fasted states at 16 weeks of age on a regular diet. The hGPR40 transgenic (47M) and nontransgenic mice were fed a high-fat diet containing 60 kcal% fat for 12 weeks. All parameters were measured in the fed and fasted state at 20 weeks of age. All values are the means ± SE, n = 7–10 per genotype. *P ≤ 0.01 vs. nontransgenic mice by Student’s t test.

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previous report suggested the involvement of GPR40 in FFA-induced lipotoxicity in \( \beta \)-cells (18), we next explored the function of GPR40 in vivo under high-fat–diet conditions. hGPR40 transgenic mice (47M) were exposed to high-fat diet (containing 60 kcal\% fat) for 9–12 weeks from 8 weeks of age. When nontransgenic mice were exposed to high-fat diet, body weight, plasma glucose, insulin, and NEFA levels in the fed state were increased compared with regular diet (Table 1). There were no obvious differences in body weight and plasma parameters in the fed state between hGPR40 transgenic and nontransgenic mice, but fasting plasma glucose levels were significantly reduced in hGPR40 transgenic mice in high-fat–diet conditions compared with those observed in regular diet conditions (Table 1). Oral glucose tolerance testing showed improved glucose tolerance (Fig. 4A and C) and augmented insulin secretion in response to glucose (Fig. 4B and D) in hGPR40 transgenic mice compared with nontransgenic mice. No apparent difference in insulin sensitivity or in epididymal adipose tissue weight was observed between hGPR40 transgenic and nontransgenic mice (Fig. 4E and F). These results indicate that improved glucose tolerance with increased insulin secretion in hGPR40 transgenic mice was maintained under conditions of high-fat–diet–induced insulin resistance.

**Improved glucose tolerance in hGPR40 transgenic mice harboring a diabetic genetic background.** The effects of overexpression of GPR40 in pancreatic \( \beta \)-cells were examined in genetically diabetic mice. Mice harboring KK background and, exhibiting obesity, glucose intolerance, and insulin resistance (25) were selected as mates, and male hGPR40 transgenic (47M) and nontransgenic mice were crossed to female mice harboring KK background. Although the mice obtained had a hybrid genetic background harboring \(~\)50% each of KK and C57BL/6J, the nontransgenic mice obtained (nontransgenic KK mice) showed heavier body weight and higher plasma insulin levels than nontransgenic C57BL/6J mice at 16 weeks of age fed with regular chow (body weight \( 28.8 \pm 0.4 \) vs. \( 38.9 \pm 0.57 \) g, plasma insulin \( 1.54 \pm 0.30 \) vs. \( 5.79 \pm 1.83 \) ng/ml for nontransgenic C57BL/6J vs. nontransgenic \( \times \) KK, respectively; \( n = 7–10 \)). hGPR40 gene expression level in islets of hGPR40 transgenic \( \times \) KK mice was \( >10 \) times higher than that of the mouse gene (Fig. 5A). The hGPR40
transgenic × KK mice did not show significant differences in body weight, plasma glucose, and insulin levels compared with nontransgenic × KK mice at 10 weeks of age (Table 2). Oral glucose tolerance testing at 12 weeks of age revealed improved glucose tolerance and increased insulin secretion in hGPR40 transgenic × KK mice compared with nontransgenic × KK mice (Fig. 5B–E). We next examined the insulin secretion in response to glucose in vitro using islets from hGPR40 transgenic × KK and nontransgenic × KK mice at 12 weeks of age. Although basal insulin secretion from islets in response to a low glucose concentration (3 mmol/l) was closely similar between hGPR40 transgenic × KK and nontransgenic × KK mice, insulin secretion against high glucose stimulation (16 mmol/l) was 3.5-fold higher in hGPR40 transgenic × KK islets compared with nontransgenic × KK islets (Fig. 5F). Stimulation with 1 mmol/l palmitate produced more enhanced insulin secretion in islets of hGPR40 transgenic × KK mice than in islets of nontransgenic × KK mice at 11 mmol/l glucose (Fig. 5G).

**Gene expression of factors regulating insulin secretion in islets isolated from hGPR40 transgenic mice.** To investigate the molecular mechanisms for enhanced insulin secretion in response to high-glucose stimulation in hGPR40 transgenic mice in vitro (Figs. 3C and D and 5F) and in vivo (Figs. 2, 4, and 5), gene expression levels of factors regulating insulin secretion were compared between islets from hGPR40 transgenic (47M) and nontransgenic mice after high-fat diet feeding for 9 weeks. The gene expression levels of insulin II, GLUT2, and glucokinase were found to be almost the same between islets of hGPR40 transgenic and nontransgenic mice (Fig. 6). We also examined the gene expression levels of UCP2, which negatively regulates insulin secretion in β-cells (26–28), and the expression level of UCP2 did not change between hGPR40 transgenic and nontransgenic mice (Fig. 6).

**DISCUSSION**

In this study, we have shown that overexpression of the hGPR40 gene using insulin II promoter resulted in improved glucose tolerance with augmented insulin secretion, and the phenotype of hGPR40 transgenic mice was not altered by high-fat–diet feeding or by gene transfer into a diabetic background. We found extremely high expression in pancreatic islets in two independent lines, but slight expression was detected in kidney in the 47M line. Both 47M and 23F hGPR40 transgenic mice showed the same phenotype, and therefore it is unlikely that the expression in kidney may affect the phenotype of hGPR40 transgenic mice. The hGPR40 transgenic mice showed slightly lower plasma glucose levels than nontransgenic mice in the fasted state but not in the fed state. These results might reflect the differential activation of GPR40 between the fasted and the fed state because natural ligands of GPR40 were FFAs (8–10), and FFA levels were significantly increased after an overnight fast in mice. In the fasted state, GPR40 may be more strongly activated than in the fed state, resulting in enhanced insulin secretion and reduced plasma glucose levels. In fact, fasting
insulin levels tended to increase more in hGPR40 transgenic mice than in nontransgenic mice.

Isolated islets from hGPR40 transgenic mice significantly secreted insulin when stimulated with palmitate, a natural ligand of GPR40, and glucose per se. Although we do not have direct evidence for GPR40 protein expression level, it was proposed that GPR40 activation by fatty acids stimulated the G\(_{\alpha_q}\)-PLC signaling pathway, involving activation of PLC and production of inositol 1,4,5-triphosphate, which leads to release of calcium from the endoplasmic reticulum (11,14 –16). Glucose and fatty acids could also augment insulin secretion through pathways involving protein kinase C (29,30). The mechanism of enhanced glucose-stimulated insulin secretion observed in hGPR40 transgenic mice islets remains unclear so far, so further studies will be needed to clarify the precise mechanism.

Steneberg et al. (18) reported that the GPR40 transgenic mice driven by the IPF-1/PDX-1 promoter had impaired \(\beta\)-cell function, hypoinsulinemia, and glucose intolerance, suggesting the involvement of GPR40 in FFA-induced toxicity in \(\beta\)-cells. In contrast, our hGPR40 transgenic mice did not develop diabetes, even after high-fat–diet feeding for 8–12 weeks. The reason for the discrepancy remains unknown. Although we may need further examination, the possible explanations are below. First is the different promoters used for the production of transgenic mice. The temporal pattern of expression of IPF-1/PDX-1 during development was different from that of insulin II (31). Transgenes of GPR40 regulated by the IPF-1/PDX-1 promoter might be expressed in pancreatic progenitors in the early embryonic stage, and these expression patterns might be influenced to the phenotype. Second, it may be caused by the difference in genetic background of each transgenic mouse. Third, the difference in GPR40 gene levels between our transgenic mice and Steneberg's mice might be a factor. In addition, our preliminary results showed that the hGPR40 transgenic mice maintained improved glucose tolerance with increased insulin secretion when fed a high-fat diet for a long period (>50

**FIG. 3. Islet structure and \(\beta\)-cell function of hGPR40 transgenic mice and nontransgenic mice.**

A: Islet morphology of hGPR40 transgenic (47M and 23F) and nontransgenic mice at 16 weeks of age. The sections were stained with anti-insulin, -glucagon, -GLUT2, and -proinsulin antibodies, respectively. B: \(\beta\)-Cell area was measured as the stained area for anti-insulin antibody. C and D: Glucose-stimulated insulin secretion in isolated islets from hGPR40 transgenic and nontransgenic mice.
E: Palmitate-stimulated insulin secretion in isolated islets from hGR40 transgenic (47M) mice and nontransgenic mice. Islets were isolated at 9 weeks from hGR40 transgenic and nontransgenic mice fed regular diet. Five islets with similar sizes from each group (four batches in each group) were used. All values are the means ± SE. **\(P < 0.01\), *\(P < 0.05\) vs. nontransgenic mice by Student's t test. NonTg, nontransgenic; Tg, transgenic. (A high-quality digital representation of this figure is available in the online issue.)
weeks), without changes in body weight and plasma parameters, including plasma insulin levels (data not shown). Therefore, our results suggest that activation of GPR40 function might not cause lipotoxicity. Latour et al. (19) and Tan et al. (20) demonstrated that islets from GPR40 knockout mice were as sensitive to fatty acid inhibition of insulin secretion on prolonged exposure as islets from wild-type animals, and they concluded that GPR40 does not play a role in the mechanisms by which fatty acids chronically impair insulin secretion. Moreover, Kebede et al. (32) reported that GPR40 knockout mice showed fasting hyperglycemia and were not protected from high-fat–diet–induced insulin resistance. Although further studies will be needed to clarify the relationship between GPR40 and lipotoxicity, these observations are consistent with our findings. Furthermore, small-molecule agonists of GPR40 have been reported (20–22), and GPR40 agonists could enhance glucose-stimulated insulin secretion and improve glucose tolerance both acutely and chronically. Results from these reports indicate that acti-

**FIG. 4.** Effects of high-fat–diet feeding on glucose homeostasis in hGPR40 transgenic mice. The hGPR40 transgenic (47M) and nontransgenic mice were fed on 60 kcal% fat diet from 8 weeks of age. Oral glucose tolerance testing was used for hGPR40 transgenic mice and nontransgenic mice. Glucose was administered orally at 1 g/kg body wt. **A and B:** Plasma glucose (A) and plasma insulin (B) at 17 weeks of age, respectively. Data in panel C represent the AUC0–120 min of plasma glucose shown in panel A, and data in panel D represent the AUC0–30 min of plasma insulin shown in panel B. **E:** Insulin tolerance test for nontransgenic and hGPR40 transgenic mice. Insulin was injected intraperitoneally at 0.5 units/kg. Plasma glucose of high-fat–diet–fed mice at 20 weeks of age. **F:** Epididymal adipose tissue weight at 20 weeks of age. All values are the means ± SE (n = 10). **P < 0.01 vs. nontransgenic mice by Student’s t test. NonTg, nontransgenic; Tg, transgenic.
FIG. 5. Improved glucose tolerance in hGPR40 transgenic mice harboring diabetic KK background on regular diet. A: Human and mouse GPR40 mRNA levels in islets from hGPR40 transgenic × KK and nontransgenic × KK mice (n = 3) by quantitative real-time PCR analyses. mRNA levels of actin were used as an internal control. B–E: Oral glucose tolerance test for hGPR40 transgenic mice and nontransgenic mice harboring hybrid background. Glucose was administered orally at 2 g/kg body wt. B and C: Plasma glucose (B) and plasma insulin (C) at 12 weeks of age on regular diet. Data in panel D represent the AUC_{0–120 min} of plasma glucose shown in panel B, and data in panel E represent the AUC_{0–30 min} of plasma insulin shown in panel C. F and G: Glucose- and palmitate-stimulated insulin secretion in isolated islets from hGPR40 transgenic × KK and nontransgenic × KK mice. Islets were isolated from regular diet–fed mice at 12 weeks. Five islets with similar sizes from each group (four batches in each group) were used. All values are means ± SE (n = 8–10). **P < 0.01, *P < 0.05 vs. nontransgenic mice by Student’s t test. NonTg, nontransgenic; Tg, transgenic.
TABLE 2
Metabolic parameters in hGPR40 transgenic mice crossed with KK mice fed regular diet

| Regular diet crossed with KK    | Body weight (g) | Plasma glucose (mg/dl) | Plasma insulin (ng/ml) |
|---------------------------------|-----------------|------------------------|------------------------|
| Nontransgenic                  | 33.8 ± 0.7      | 200.1 ± 5.8            | 2.18 ± 0.58            |
| Transgenic                     | 33.3 ± 0.5      | 208.5 ± 5.9            | 3.65 ± 0.76            |

The hGPR40 transgenic (47M) mice harboring C57BL/6J and KK background were used for analyses. All parameters were measured in the fed state at 10 weeks of age. All values are the means ± SE; n = 8–10 per genotype.

vation of GPR40 might be beneficial for glucose control in type 2 diabetes without lipotoxicity, and the phenotypes of our hGPR40 transgenic mice strongly support this conclusion.

Obesity commonly induces insulin resistance and causes an increase in the requirement of insulin secretion from the pancreas, and the enhancement of this phenomenon further exacerbates obesity and insulin resistance. The molecular mechanism of this phenomenon is unclear, but it may be related to increased production and secretion of nonesterified fatty acids and metabolically harmful adipokines by insulin-resistant adipocytes during obesity (33–35). In our results, hGPR40 transgenic mice fed a high-fat diet did not show significant differences in body weight, adipose tissue weight, and plasma insulin level. Moreover, when insulin resistance was reinforced by crossing with KK background–harboring mice, hGPR40 transgenic × KK mice did not show significant differences in body weight and plasma insulin level from nontransgenic × KK mice. Therefore, activation of GPR40 may not cause sustained hyperinsulinemia but may enhance glucose-stimulated insulin secretion essentially in the prandial period.

The M3 muscarinic acetylcholine receptor subtype couples with Goq in pancreatic β-cells (36–37). It has been reported that overexpressed M3 muscarinic receptors in pancreatic β-cells showed almost the same phenotype as the hGPR40 transgenic mice (38), and the potentiation of Goq signaling in pancreatic β-cells may lead to the main-

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REFERENCES
1. Unger RH. The physiology of cellular liporegulation. Annu Rev Physiol 2003;65:333–347
2. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. Diabetesologia 1999;42:128–138
3. Poitout V, Robertson RP. Minireview: secondary beta-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity. Endocrinology 2002;143:339–342
4. Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF. Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. J Clin Invest 1992;90:320–325
5. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A 1998;95:2498–2502
6. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE. Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. J Biol Chem 1992;267:5802–5810
7. Yaney GC, Corkey BE. Fatty acid metabolism and insulin secretion in pancreatic beta cells. Diabetologia 2003;46:1307–1312
8. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogli K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu N, Matsumura F, Noguchi Y, Shinozuka H, Hinuma S, Fujisawa Y, Fujino M. Fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature 2003;422:173–176
9. Briscoe CP, Talayyon M, Andrews JL, Benson WG, Chambers JK, Eliert MM, Ellis C, Elshourbagy NA, Goetz A, Minnick DT, Murdock PR, Sauls HR, Jr, Shabon U, Spinage LD, Strum JC, Szeckeres PG, Tan KB, Way JM, Ignar DM, Wilson S, Muir AL. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem 2003;278:11308–11311
10. Kotarsky K, Nilsson NE, Flodgren E, Owmam C, Olde B. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. Biochem Biophys Res Commun 2003;301:406–410
11. Fujimura K, Maekawa F, Yada T. Oleic acid interacts with GPR40 to induce Ca2+ signaling in rat islet beta-cells: mediation by PLC and L-type Ca2+ channel and link to insulin release. Am J Physiol Endocrinol Metab 2005;289:E670–E677
12. Tomita T, Masuzaki H, Noguchi M, Iwakura H, Fujikura J, Tanaka T, Ebihara K, Kawamura J, Komoto I, Kawaguchi Y, Fujimoto K, Doi R, Shimada Y, Hosoda K, Imamura M, Nakao K. GPR40 gene expression in human pancreas and insulinoma. Biochem Biophys Res Commun 2005;338:1788–1790
13. Tomita T, Masuzaki H, Iwakura H, Fujikura J, Noguchi M, Tanaka T, Ebihara K, Kawamura J, Komoto I, Kawaguchi Y, Fujimoto K, Doi R, Shimada Y, Hosoda K, Imamura M, Nakao K. Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic beta cells and implications for insulin secretion. Diabetesologia 2006;49:962–968
14. Brown AJ, Jupe S, Briscoe CP. A family of fatty acid binding receptors. DNA Cell Biol 2005;24:54–61
15. Shapiro H, Shachar S, Sekler I, Hershfield M, Walker MD. Role of GPR40 in fatty acid action on the beta cell line INS-1E. Biochem Biophys Res Commun 2005;335:97–104
16. Gromada J. The free fatty acid receptor GPR40 generates excitement in pancreatic beta-cells. Endocrinology 2006;147:672–673
17. Schnell S, Schaefer M, Schoff C. Free fatty acids increase cytosolic free calcium and stimulate insulin secretion from beta-cells through activation of GPR40. Mol Cell Endocrinol 2007;263:173–180
18. Steneberg P, Rubins N, Bartooov-Shifman R, Walker MD, Edlund H. The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. Cell Metab 2005;1:245–258
19. Latour MG, Alquier T, Oseid E, Tremblay C, Jetton TL, Liao J, Lin DC, Poitout V. GPR40 is necessary but not sufficient for fatty acid stimulation of insulin secretion in vivo. Diabetes 2007;56:1087–1094
20. Tan CP, Feng Y, Zhou YP, Eiermann GJ, Petrov A, Zhou C, Lin S, Salituro G, Meinke P, Mosley R, Akiyama TE, Einstein M, Kumar S, Berger JP, Mills SG, Thornberry NA, Yang Y, Howard AD: Selective small-molecule agonists of G protein-coupled receptor 40 promote glucose-dependent insulin secretion and reduce blood glucose in mice. Diabetes 57:2211–2219, 2008
21. Briscoe CP, Peat AJ, McKeown SC, Corbett DF, Goetz AS, Littleton TR, McCoy DC, Kenakin TP, Andrews JL, Ammala C, Forwald JA, Ignar DM, Jenkinson S. Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. Br J Pharmacol 2006;148:619–628
22. Song F, Lu S, Gunnet J, Xu JZ, Wines P, Proost J, Liang Y, Baumann C, Lenhard J, Murray WV, Demarest KT, Kuo GH. Synthesis and biological evaluation of 3-aryl-3-(4-phenoxy)-propionic acid as a novel series of G protein-coupled receptor 40 agonists. J Med Chem 2007;50:2807–2817
23. Nagy A. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2003
24. Sutton R, Peters M, McShane P, Gray DW, Morris PJ. Isolation of rat pancreatic islets by ductal injection of collagenase. Transplantation 1986;42:689–691
25. Takeomi S, Ikeda H. KK and KKAy mice. In Animal Models of Diabetes. A Primer. Shima AAF, Shafir E, Eds. Amsterdam, the Netherlands: Harwood Academic Publishers, 2001, p. 129–142
26. Lameloise N, Muzzin P, Prentki M, Assimacopoulos-Jeannet F. Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion? Diabetes 2001;50:803–809
27. Zhang CY, Bafy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. Cell 2001;105:745–755
28. Chan CB, De Leo D, Joseph JW, McQuaid TS, Ha XF, Xu F, Tsushima RG, Pennefather PS, Salapatek AM, Wheeler MB. Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. Diabetes 2001;50:1302–1310
29. Yaney GC, Korshak HM, Corkey BE. Long-chain acyl CoA regulation of protein kinase C and fatty acid potentiation of glucose-stimulated insulin secretion in clonal beta-cells. Endocrinology 2000;141:1989–1998
30. Alcazar O, Quy-yue Z, Gine E, Tamarit-Rodriguez J. Stimulation of islet protein kinase C translocation by palmitate requires metabolism of the fatty acid. Diabetes 1997;46:1153–1158
31. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 1996;122:985–995
32. Kebede M, Alquier T, Latour MG, Semache M, Tremblay C, Poitout V. The fatty-acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. Diabetes 2008;57:2432–2437
33. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997;46:3–10
34. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
35. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science 1996;274:1185–1188
36. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. Diabetes 2004;53:1714–1720
37. Gromada J, Hughes TE. Ringing the dinner bell for insulin: muscarinic M3 receptor activity in the control of pancreatic beta cell function. Cell Metab 2006;3:390–392
38. Gautham D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH, Cui Y, Mears D, Lu H, Deng C, Heard T, Weiss J. A critical role for beta cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. Cell Metab 2006;3:449–461