Puerarin Protects Pancreatic β-Cells in Obese Diabetic Mice via Activation of GLP-1R Signaling

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Diabetes is characterized by a loss and dysfunction of the β-cell. Glucagon-like peptide 1 receptor (GLP-1R) signaling plays an important role in β-cell survival and function. It is meaningful to identify promising agents from natural products which might activate GLP-1R signaling. In this study, puerarin, a diet isoflavone, was evaluated its beneficial effects on β-cell survival and GLP-1R pathway. We showed that puerarin reduced the body weight gain, normalized blood glucose, and improved glucose tolerance in high-fat diet-induced and db/db diabetic mice. Most importantly, increased β-cell mass and β-cell proliferation but decreased β-cell apoptosis were observed in puerarin-treated diabetic mice as examined by immunostaining of mice pancreatic sections. The protective effect of puerarin on β-cell survival was confirmed in isolated mouse islets treated with high glucose. Further mechanism studies showed that the circulating level of GLP-1 in mice was unaffected by puerarin. However, puerarin enhanced GLP-1R signaling by up-regulating expressions of GLP-1R and pancreatic and duodenal homeobox 1, which subsequently led to protein kinase B (Akt) activation but forkhead box O1 inactivation, and promoted β-cell survival. The protective effect of puerarin was remarkably suppressed by Exendin(9–39), an antagonist of GLP-1R. Our study demonstrated puerarin improved glucose homeostasis in obese diabetic mice and identified a novel role of puerarin in protecting β-cell survival by mechanisms involving activation of GLP-1R signaling and downstream targets. (Molecular Endocrinology 30: 361–371, 2016)

Loss of β-cell and the impaired function of β-cell might be the important pathogenesis of diabetes. The underlying mechanisms of β-cell failure in type 2 diabetes mellitus (T2DM) are complex and still largely unknown. Lipo- and glucotoxicity, subclinical inflammation, oxidative stress, and endoplasmic reticulum (ER) stress may play an important role in these processes (1, 2). Increase the β-cell mass and restore the function of β-cell become potential strategies for diabetes treatment. The incretin hormone glucagon-like peptide 1 (GLP-1) is an important target for diabetes therapy because of its ability to potentiate glucose-stimulated insulin secretion (GSIS) as well as to promote β-cell proliferation and survival (3). GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-IV (DPP-4) inhibitors have been developed as new therapies based on these incretin effects.

In the treatment of metabolic syndrome, the traditional Chinese medicine is an excellent alternative and

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Abbreviations: Akt, protein kinase B; DPP-4, dipeptidyl peptidase-IV; DAPI, 4′,6-diamidino-2-phenylindole; DMSO, dimethyl sulphoxide; Foxo1, forkhead box o1; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; GSIS, glucose-stimulated insulin secretion; HFD, high-fat diet; IPGTT, ip glucose tolerance test; Ki67, MKI67; KRB, Krebs-Ringer bicarbonate buffer; ND, normal diet; Pdx-1, pancreatic and duodenal homeobox 1; STZ, streptozotocin; TCF7L2, T-cell factor 7-like 2; T2DM, type 2 diabetes; TUNEL, Transferase-mediated dUTP nick end labeling; Wnt, wingless-type MMTV integration site; WT, wild type.
complementary medicine with a long history. Recently, some natural products have been shown effects on regulating GLP-1 level. For example, it was reported that a naturally derived agent, berberine, promoted GLP-1 secretion in streptozotocin (STZ)-induced diabetic rats (4). A latest study showed that the ethyl acetate fraction of radix of *Acorus calamus* L. lowered blood glucose levels in STZ-induced mice and *db/db* mice via elevated GLP-1 secretion and *gcg* mRNA level (5).

The popularity of dietary natural product supplements is increasing worldwide even in Western countries. Puerarin, a diet isoflavone, is found in a number of herbs, such as the root of *Radix puerariae* (kudzu root), which is a nutritious food ingredient in East Asia traditionally. In Traditional Chinese Medicine, *R. puerariae* has been used for diabetic treatment for hundreds of years. Studies have shown that antidiabetic effects of puerarin are mediated by antioxidative (6), modulating lipid metabolism (7), and improving insulin secretion and resistance (8). A recent publication reported that puerarin inhibited β-cell death in STZ-induced diabetic mice, and its effect was mediated by the phosphoinositide 3-kinase (PI3K)/ AKT), protein kinase B, pathway (9). However, impacts of puerarin on β-cell function and proliferation have not been extensively investigated so far. The underlying mechanisms involved need to be assessed further.

The main goal for diabetes therapy is to prevent depletion of existing β-cells, as well as promoting new β-cell formation. In the present study, high-fat diet (HFD)-induced and *db/db* diabetic mice were applied to evaluate the antidiabetic effect of puerarin. Here, we aimed to investigate the direct effects of puerarin on β-cell proliferation and apoptosis in condition of hyperglycemia in vivo and in vitro. Effects of puerarin on GLP-1R signaling were examined for the first time in our study.

### Materials and Methods

#### Reagent

Puerarin (purity > 98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Exendin-4 and Exendin(9–39) were from Sigma.

#### Animals

All animal experiments were conducted in accordance with Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and approved by the Research Animal Care Committee of Nanjing Medical University. All animals were housed in a temperature-controlled room with a 12-hour light, 12-hour dark cycle and were allowed free access to food and water during the course of experiments. Four-week-old male C57BL/6J mice (SLAC Laboratory Animals) were fed a HFD (with 60 kcal% fat, D12492; Research Diets) or normal chow diet. Puerarin solution was prepared in 0.5% CMC-Na and delivered by oral gavage at dosage of 150 mg/kg daily. Puerarin intervention (150 mg/kg) was initiated after 12 weeks of the HFD and continued for 35 days. Four-week-old male C57BL/6J (BKS) mice and BKS.Cg-Dock7m*+/+, Lepr*db/J*Nju (db/db) mice were obtained from Model Animal Research Center of Nanjing University. Puerarin was given for 55 days in *db/db* mice. The control group was given vehicle. After 35 or 55 days of puerarin administration, ip glucose tolerance test (IGT) was performed, and blood samples and pancreas were collected for subsequent analysis. A total of 12 mice in each group were used.

#### Intraperitoneal glucose tolerance tests

Mice were fasted 12 hours overnight and injected ip with glucose at a dose of 2 mg/g body weight. Blood samples were obtained at time points 0, 30, 60, 90, and 120 minutes for glucose measurements using a glucometer (Accu-Chek Active; Roche, Inc).

#### Blood parameters

Blood samples were collected from random-fed mice and centrifuged at 3000 rpm for 10 minutes at 4°C, and the serum was frozen until analysis. Diprotin A (DPP-4 inhibitor) and aprotinin were added to each blood sample to final concentrations of 100 μM and 85 μg/mL, respectively. Insulin was determined using a mouse insulin ELISA kit (Alpco). Total cholesterol and triglycerol were assessed by assay (Nanjing Jiancheng). Serum adiponectin and leptin were assessed by mouse ELISA kit (Boster). GLP-1 was measured using a mouse/rat specific GLP-1(7–36) ELISA assay (Phoenix). All procedures were conducted according to the manufacturer’s instructions.

#### Analysis of β-cell mass

β-Cell mass was measured as previously described (10). In brief, pancreatic sections were stained with antimouse insulin antibody (ab7842; Abcam) and scanned by a Nikon MEA53200 (Nikon) microscope. The cross-sectional areas of pancreas and β-cells were determined by NIS-Elements software (Nikon). β-Cell mass/pancreas was estimated by the product of the relative cross-sectional area of β-cells per total tissue and the weight of the pancreas.

#### Mouse pancreatic islets isolation and culture

Mouse islets were isolated from C57BL/6J mice (SLAC Laboratory Animals) by common bile duct perfusion using collagenase type 4 (Worthington) as described previously (11) and cultured in RPMI 1640 containing 11.1 mmol/L glucose, 100-U/mL penicillin, 100-mg/mL streptomycin, and 10% fetal bovine serum (FBS) (Invitrogen). For treatment of islets, medium was changed to culture medium containing 33.3mM glucose, treated with dimethyl sulphoxide (DMSO) as control or with puerarin (50μM), or Exendin-4 (50nM), or Exendin(9–39) (200nM) for 3 days.

#### Min6 cell culture

Min6 cells were obtained from American Type Culture Collection and maintained in 5mM glucose DMEM, supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 50 mmol/L
β-mercaptoethanol, 100-U/mL penicillin, and 0.1-mg/mL streptomycin in 5% CO2 at 37°C.

**Glucose-stimulated insulin secretion**

For acute insulin release, islets were washed and preincubated (30 min) in Krebs-Ringer bicarbonate buffer (KRB) containing 2.8mM glucose. The KRB was then replaced by KRB containing 2.8mM glucose for 1 hour (basal), followed by additional 1 hour of incubation in KRB containing 16.7mM glucose (stimulated). For insulin content, cells were extracted with 0.18N HCl in 70% ethanol. Insulin was determined using a mouse insulin ELISA kit (Alpco).

**Immunofluorescence staining**

Pancreatic tissue and cultured mouse islets were processed as previously described (12). Sections were fixed with 4% paraformaldehyde followed by permeabilization with 0.5% Triton X-100. Four-micrometer paraffin sections of mouse pancreases deparaffinized, rehydrated, antigen unmasking. Then islets or sections were incubated overnight at 4°C with antiinsulin (ab7842; Abcam), anti-Ki67 (MKi67) (BD Pharmingen), antibodies followed by fluorescein isothiocyanate (FITC)- or Cyanine 3 (Cy3)-conjugated secondary antibodies (Jackson ImmunoResearch). β-Cell apoptosis in pancreatic sections was analyzed by the transferase-mediated dUTP nick end labeling (TUNEL) staining kit (in red) (In Situ Cell Death Detection kit, analyzed by the transferase-mediated dUTP nick end labeling). Ki67 and TUNEL staining were analyzed using another TUNEL kit (In Situ Cell Death Detection kit, AP; Roche Diagnostics). Slides were mounted with Vectashield with 4,6-diamidino-2-phenylindole (DAPI) (Vector Labs). Ki67 and TUNEL staining were analyzed by puerarin treatment in multiple group comparisons.

**Results**

**Puerarin normalized hyperglycemia and improved glucose tolerance in db/db and HFD diabetic mice**

Based on previous reports (7, 8), we tried 2 doses of puerarin (chemical structure shown in Figure 1A), which were 150 and 300 mg/kg in preliminary study. Both of them showed significant glucose-lowering effects as compared with vehicle (data not shown). In the present study, we chose the dose of 150 mg/kg for subsequent experiments.

To assess the ability of puerarin to prevent progression of diabetes, we initiated puerarin treatment in 4-week db/db mice, which are prediabetic. The control group was given vehicle. During the whole 55-day experiment, body weight gain of db/db mice treated by puerarin was lower than that of the corresponding vehicle-treated db/db mice (Figure 1B). The vehicle db/db group developed to be diabetes at 6 weeks of age, and fasting blood glucose levels continued to increase over time (Figure 1C). In the first 15 days, puerarin did not strongly prevent progressing hyperglycemia in db/db mice. However, db/db mice were protected from developing such severe hyperglycemia by puerarin after 20 days of treatment, and this effect was maintained on subsequent days compared with vehicle-treated db/db mice. After the 55-day treatment, experiments of ip glucose challenge (IPGTT) were performed in these 12-week mice (Figure 1D). The response to IPGTT was seriously impaired in db/db mice which resulted in huge increased glucose levels compared with wild-type (WT) mice after glucose injection. Puerarin-treated db/db mice were kind of protected from such a severe glucose intolerance compared with vehicle-treated db/db mice. However, db/db mice were used to develop severe mild glucose intolerance, as compared with WT mice.

In parallel, another obese T2DM mouse model, 12-week HFD-induced diabetic mice were treated with puerarin for 35 days. Similarly, the body weight gain of HFD mice was deducted by puerarin significantly (Figure 1E). HFD feeding induced a marked increase in fasting
blood glucose level in HFD mice compared with the normal diet (ND) mice (Figure 1F). Puerarin displayed hypoglycemic effect on HFD mice after 10 days of treatment compared with the vehicle, and this effect continued until the end of the experiment. In addition, the improvement of IPGTT in 17-week HFD mice with puerarin administration was observed as well (Figure 1G).

**Puerarin increased β-cell mass and promoted β-cell survival without changing GLP-1 levels in db/db and HFD diabetic mice**

Various natural products that increase plasma insulin levels and exert hypoglycemic effects in db/db mice have been reported (13–15). Here, we noticed the fasting serum insulin levels in 12-week db/db mice were decreased compared with WT mice, whereas puerarin significantly elevated the insulin levels in db/db mice compared with vehicle (Figure 2A). Similar increase of insulin level was observed in puerarin-treated 17-week HFD mice (Figure 2B).

Immunostaining for β-cells in pancreatic sections by insulin showed a significant reduction of β-cell mass in 12-week db/db and 17-week HFD mice vehicle treated (Figure 2, C and D). In contrast, the puerarin-treated group exhibited induction of β-cell mass (1.4-fold increase in db/db mice and 1.5-fold increase in HFD, compared with corresponding vehicle-treated mice), which may partially account for the increase of insulin level as we observed.

The increase in β-cell mass induced by puerarin raised the possibility that this compound may stimulate β-cell proliferation or prevent depletion of existing β-cells. Next, we investigated the impact of puerarin on β-cell proliferation by Ki67/insulin immunostaining in pancreatic sections (Figure 2E). Positive Ki67 staining of β-cells was observed in pancreatic sections of ND and WT mice but was rarely detected in HFD and db/db mice. In contrast, positive Ki67 staining was found in HFD and db/db mice treated with puerarin (2.05-fold increase in HFD and 2.25-fold increase in db/db mice, compared with vehicle, respectively).

In addition, we examined β-cell apoptosis by TUNEL/insulin immunostaining in pancreatic sections as well (Figure 2F). TUNEL-positive β-cell was barely found in sections from both ND groups and from WT group. Only 0.1% and 0.07% of β-cells were detected to be TUNEL-positive. The β-cell apoptosis was strongly increased in HFD and db/db mice (0.76% and 1.85%, respectively), which could be significantly suppressed by puerarin (2.1-fold reduction in HFD and 3.5-fold reduction in db/db mice, respectively, compared with vehicle).
These observations suggest that puerarin might exert a glucose lowering effect by protecting β-cells from damages caused by hyperglycemia. The incretin hormone GLP-1 is a key regulator to maintain glucose homeostasis by increasing insulin secretion as well as promoting β-cell survival (3). We wondered whether the effect of puerarin on β-cell survival is resulted from increase of GLP-1 level. As shown in Figure 2G, the circulating levels of active GLP-1 in db/db mice and HFD mice were increased compared with WT or ND mice, respectively. However, puerarin failed to elevate GLP-1 levels significantly despite its potential β-cell protective effects.

**Puerarin improved β-cell function and up-regulating GLP-1R expression**

GLP-1 acts through the cognate G protein-coupled receptors GLP-1R, which are expressed in several tissues, including pancreatic islets (16). In next study, impacts of puerarin on expression of GLP-1R and activation of the downstream targets were detected in isolated mouse islets cultured in high-glucose condition to mimic the hyperglycemia in vivo.

Results of RT-PCR from islets displayed that GLP-1R mRNA level was decreased by high glucose, whereas it was remarkably increased up to 7.5-fold by puerarin compared with the DMSO treatment (Figure 3A). Pdx-1 is a transcription factor that critically regulates β-cell specific genes such as insulin, glucose transporter 2 expressions and multiple aspects of β-cell function and survival. mRNA levels of Pdx-1 and Ins1 were induced by puerarin to 4.8- and 3.1-fold, respectively, compared with the DMSO treatment (Figure 3A). Interesting, here, we found that puerarin could up-regulate TCF7L2 mRNA level with 3.4-fold (to the DMSO) (Figure 3A). TCF7L2 is an important transcription factor of wingless-type MMTV integration site (Wnt)/β-catenin signaling, which is a key modulator for β-cell survival and regeneration (17, 18).
Meanwhile, GSIS assay revealed that the insulin secretion of islets was strongly impaired by 33.3mM glucose compared with 11.1mM glucose incubations. Puerarin, however, induced a 1.8-fold increase in the stimulatory index, as compared with DMSO treated (Figure 3B).

Simultaneously, we measured the GLP-1R and Pdx-1 expressions in treated islets by Western blotting (Figure 3C). High glucose decreased GLP-1R as well as Pdx-1 expressions while inducing caspase3 cleavage, which triggered to β-cell apoptosis. Confirmed with RT-PCR results, puerarin significantly restored the GLP-1R and Pdx-1 expressions but reduced caspase3 cleavage to preserve β-cell survival.

A recent report showed that chronic hyperglycemia could lead to the loss of the GLP-1R from the cell surface and an impairment of GLP-1R signaling (19). Here, we examined the GLP-1R expression at the plasma membrane and cytoplasm, respectively, in Min6 cells with 33.3mM glucose treatment (Figure 3D). GLP-1R level at the membrane and the membrane to cytosolic ratio of GLP-1R were remarkably deducted by high glucose. Again, puerarin up-regulated the expression of GLP-1R and recovered the membrane distribution of GLP-1R.

Puerarin promoted β-cell function and survival via GLP-1R signaling activation

Antiapoptotic mechanisms of GLP-1 are mediated through activation of Akt (20). Akt is a key molecule of PI3K/Akt pathway that regulates glucose metabolism and β-cell survival. In view of the fact that puerarin could up-regulate GLP-1R expression, we detected activation of Akt and Foxo1, which are downstream targets of GLP-1R signaling (Figure 4A). As a member of the forkhead transcription family, the action of Foxo1 is suppressed when phosphorylated by Akt (21). As expected, puerarin activated Akt but inactivated Foxo1 significantly by inducing Akt and Foxo1 phosphorylations in islets treated by high glucose compared with DMSO control. Interesting, Exendin(9–39), an antagonist of GLP-1R, showed the strong ability to inhibit the Akt activation and Foxo1 inactivation induced by puerarin.

To explore the role of GLP-1R signaling in effects of puerarin, we performed GSIS assay in mouse islets with different treatments. As shown in Figure 4B, the insulin stimulatory effect of Exendin-4, a well-known agonist of GLP-1R, was significantly enhanced by puerarin. However, the insulin stimulatory effect of puerarin was inhibited by Exendin(9–39). Exendin(9–39) itself alone di-
played an impairment of insulin secretion compared with control, but there was no significant difference, which was consistent with other report (22).

Further, cultured isolated mouse islets were applied to confirm the protective effects of puerarin on β-cell survival detected by immunostaining with Ki67 and TUNEL (Figure 4C). In normal condition (11.1mM glucose), puerarin itself showed an ability to stimulate the β-cell proliferation (1.39-fold increased by puerarin compared with DMSO treated). Elevated glucose level (33.3mM) re-

Figure 4. Puerarin promoted β-cell function and survival depended on GLP-1R signaling activation. A, Representative Western blottings to show the activation of GLP-1R signaling in the islets cultured in 33.3mM glucose with different treatments, puerarin (50μM), or Exendin(9–39) (Ex-39) (200nM), DMSO as control. The densitometric analyses of 3 independent experiments are shown; *, P < .05; **, P < .01. B, GSIS assay was performed in treated mouse islets with different treatments, puerarin (50μM), or Exendin-4 (Ex-4) (50nM), or Ex-39 (200nM), DMSO as control. Stimulatory index denotes the amount of stimulated divided by the amount of basal insulin secretion. Data are shown as mean ± SE from 3 independent experiments. C, Isolated mouse islets were exposed to different conditions for 3 days. Proliferation was measured by the Ki67 staining (in red, indicated by white arrows), and apoptosis by the TUNEL assay stained in black (indicated by black arrows). Islets were triple stained for insulin in green and counterstained for DAPI in blue. Scale bars, 20 μm. Results are expressed as mean ± SE of the percentage of Ki67-positive or TUNEL-positive β-cells; *, P < .05; **, P < .01.
duced β-cell proliferation while inducing β-cell apoptosis, compared with control (11.1mM glucose). On the contrary, islets treated with puerarin were protected against the deleterious effects of high glucose. Proliferation of β-cell was 2.3-fold increased by puerarin compared with DMSO treated, whereas apoptosis was 2.1-fold decreased accordingly. Moreover, the protective effects of puerarin on β-cell survival were significantly suppressed by Exendin(9–39) (2.1-fold decrease in proliferation while 1.66-fold increase in apoptosis). Here, we noticed that Exendin(9–39) had no significant effect on β-cell proliferation but rather increased β-cell apoptosis.

**Puerarin presented beneficial lipid metabolic effects in HFD and db/db mice**

Hyperglycemia and insulin resistance give rise to diabetic dyslipidemia in T2DM. It is well known that GLP-1 could also modulate the lipid metabolism as a key regulator (23). HFD and db/db mice are both obese diabetic animal models with elevated total cholesterol and triglyceride contents. We found that puerarin decreased cholesterol and triglyceride levels significantly in db/db mice (Figure 5, A and B). In HFD mice, puerarin reduced total cholesterol significantly but had no remarkable effect on triglyceride levels (Figure 5, C and D). Recent studies suggest that adipokines can play a beneficial role in various metabolic diseases, especially in T2DM. Two important adipokines, adiponectin and leptin were measured as well. Puerarin up-regulated circulating adiponectin level in db/db mice, which was decreased in vehicle db/db group compared with WT mice (Figure 5E), whereas no significant change of adiponectin was found in HFD mice (Figure 5F). However, the induction of leptin in HFD mice was significantly reduced by puerarin compared with vehicle group (Figure 5G). These data indicated that the beneficial lipid metabolic effects of puerarin in HFD and db/db mice might be associated with the activation of GLP-1R pathway by puerarin.

**Discussion**

Healthy β-cells can compensate for insulin resistance by increasing in number and functional output. Pancreatic β-cell replication plays a primary role in keeping β-cell mass (24). However, β-cell mass is decreased in patients with diabetes mellitus, suggesting a primary role for β-cell depletion in the pathogenesis of T2DM (25). In this study, increased β-cell apoptosis and decreased β-cell proliferation were obtained in 17-week HFD mice and 12-week db/db mice, which might be resulted from long-term high-glucose toxicity and lipotoxicity. Significant induction of β-cell apoptosis in db/db mice was also reported by other study (26).

A variety of animal models of T1DM and T2DM have been used for diabetes-related researches, each with their own characteristics. STZ-induced type 1 diabetic mice were employed commonly in studies of puerarin antidiabetic effects (8, 9). However, STZ mice are not appropriate animal model for T2DM study. Few studies have assessed antidiabetic effects of puerarin in obese diabetic mice models so far. Growing evidence has shown obesity is a risk for T2DM, which might induce insulin resistance as well as impair β-cell function and survival (27). In the present study, we applied HFD mice and db/db mice to evaluate the puerarin antidiabetic effect, which are 2 commonly used obese diabetic mouse models for T2DM study (28). Four-week db/db mice start to display metabolic disorders and naturally develop diabetes by 6–8 weeks age. Severe depletion of the β-cells is observed in these
mice with aging. As the diet-induced obesity mouse model, feeding a HFD in C57Bl/6 mice resulted in hyperinsulinemia and impaired glucose tolerance after 4 weeks, elevation of fasting glucose after 8 weeks, hyperglycemia, and loss of GSIS after 12 weeks of the HFD. β-Cell apoptosis is increased after 16 weeks of the HFD. Thus, HFD-induced diabetic mice and db/db mice applied in our study were nice models for β-cell survival study.

Herbal medicine as one of the most popular alternative medicines has a key role in the treatment of diabetest and other metabolic diseases in many developing countries. More than two-thirds of the active agents of drugs have relationship to natural sources (29). A large number of herbs and extracts have been shown effects on preventing metabolic disorders like diabetes and obesity with different mechanisms (30). Some natural components have functions on improving β-cell proliferation. For instance, genistein, a flavonoid in legumes and some herbal medicines, directly modulates pancreatic β-cell proliferation and function via activation of the cAMP/protein kinase A (PKA)-dependent ERK1/2 signaling pathway (31). Here, we demonstrated that puerarin could promote β-cell proliferation in HFD and db/db mice in vivo detected by Ki67/insulin costaining. Meanwhile, the β-cell apoptosis could be significantly suppressed by puerarin measured by TUNEL staining in mice pancreatic sections. More β-cell proliferation and less β-cell apoptosis contributed together to the increase of β-cell mass by puerarin in HFD and db/db mice.

The GLP-1R signaling controls the physiological response to GLP-1 and is currently a major target for the development of therapeutics owing to the broad range of potential beneficial effects in T2DM. These include promotion of glucose-dependent insulin secretion, increased insulin biosynthesis, preservation of β-cell mass, improved peripheral insulin sensitivity, and promotion of weight loss (32). The decrease of GLP-1R expression induced by high glucose was found in this study which was confirmed to our previous report (12). In line with our data, a decrease in GLP-1R and GIP-R has been observed in response to hyperglycemia, significant reductions occurring in islets from 90% pancreatectomized hyperglycemic rats as well as in isolated rat islets cultured in high glucose for 48 hours (33). Another study reported that the expressions of GLP-1R, gastric inhibitory polypeptide receptor (GIPR), and peroxisome proliferator-activated receptor α (PPARα) were down-regulated when INS-1 cells were treated with glucose, whereas their expressions were up-regulated when treated with metformin (34). Therefore, besides GLP-1R agonists and DPP-4 inhibitors, recovery the GLP-1R expression itself and enhance the GLP-1R signaling activation might be an alternative strategy for diabetic treatment.

The present study revealed that puerarin rescued the β-cell failure and promoted β-cell proliferation through up-regulating GLP-1R expression and activating its downstream target Akt, which led to inactivation of Foxo1 and caspase3 subsequently. Foxo1 acts as a transcription factor to inhibit Pdx-1 activity and mediate β-cell dysfunction and apoptosis. The increase of Pdx-1 by puerarin was obtain as well. The protective effect of puerarin was remarkably suppressed by Exendin(9–39), an antagonist of GLP-1R, which indicated that the function of puerarin is GLP-1R dependent. It is well known that mRNA is not a direct indication of protein level. Different regulation mechanisms (such as synthesis and degradation rates), acting on both the synthesized mRNA and the synthesized protein, affect the amount of the 2 molecules differentially (35). That is why the changes in protein expressions of GLP-1R and Pdx-1 shown in our study were not exactly correlated with changes in mRNA levels.

Interesting, here, we noticed that puerarin up-regulated the TCF7L2 expression as well. As a major transcription factor of Wnt signaling, studies have showed that Wnt/TCF7L2 pathway interacts with GLP-1 signaling. TCF7L2/β-catenin fosters synthesis of GLP-1 in intestinal L cells (36). Liu and Habener (37) reported that GLP-1 and Exendin 4 activated TCF7L2-dependent Wnt signaling to enhance β-cell proliferation. Wnt/TCF7L2 was able to improve β-cell turnover and function (38) (39). One new publication presented that TCF7L2 could positively regulate expressions of transcription factors like musculoaponeurotic fibrosarcoma oncogene family A and Pdx-1, which are crucial for β-cell regeneration (40). Here, we observed the increasing of TCF7L2 and Pdx-1 expressions induced by puerarin. These observations implied modulating effects of puerarin on Wnt signaling which need to be investigated in our future study.

In conclusion, we identified a novel role of puerarin in β-cell survival by mechanisms involving the activation of GLP-1R signaling. Our finding highlights the potential value of puerarin as a possible treatment of T2DM.

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