Effects of the Antitumor Drug OSI-906, a Dual Inhibitor of IGF-1 Receptor and Insulin Receptor, on the Glycemic Control, β-Cell Functions, and β-Cell Proliferation in Male Mice

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The IGF-1 receptor has become a therapeutic target for the treatment of cancer. The efficacy of OSI-906 (linstinib), a dual inhibitor of IGF-1 receptor and insulin receptor, for solid cancers has been examined in clinical trials. The effects of OSI-906, however, on the blood glucose levels and pancreatic β-cell functions have not yet been reported. We investigated the impact of OSI-906 on glycemic control, insulin secretion, β-cell mass, and β-cell proliferation in male mice. Oral administration of OSI-906 worsened glucose tolerance in a dose-dependent manner in the wild-type mice. OSI-906 at a dose equivalent to the clinical daily dose (7.5 mg/kg) transiently evoked glucose intolerance and hyperinsulinemia. Insulin receptor substrate (IRS)-2-deficient mice and mice with diet-induced obesity, both models of peripheral insulin resistance, exhibited more severe glucose intolerance after OSI-906 administration than glucokinase-haploinsufficient mice, a model of impaired insulin secretion. Phloridzin improved the hyperglycemia induced by OSI-906 in mice. In vitro, OSI-906 showed no effect on insulin secretion from isolated islets. After daily administration of OSI-906 for a week to mice, the β-cell mass and β-cell proliferation rate were significantly increased. The insulin signals in the β-cells were apparently unaffected in those mice. Taken together, the results suggest that OSI-906 could exacerbate diabetes, especially in patients with insulin resistance. On the other hand, the results suggest that the β-cell mass may expand in response to chemotherapy with this drug. **(*Endocrinology* 155: 2102–2111, 2014)**

Type 2 diabetes has been linked to increased rates of cancer incidence and mortality (1, 2). Many factors involved in the progression of diabetes are also associated with the incidence of cancer, including advanced age, sex, hyperglycemia, dyslipidemia, hyperinsulinemia, and low-grade inflammation. Hence, the development of therapeutic strategies for cancer that will not worsen type 2 diabetes is necessary.

Insulin and IGF-1 receptor signaling stimulates cell proliferation and migration of many cells, including the pancreatic β-cells (3–5). Insulin and the IGF receptor are also expressed in most cancer cells, and activation of the receptors in these cells induces proliferation of the cancer cells and development of metastasis. IGF-1 exerts more potent mitogenic and antiapoptotic activities than insulin (6). For this reason, a number of anti–IGF-1 receptor drugs, including monoclonal antibodies and tyrosine kinase inhibitors have been developed. OSI-906 (linstinib) is an orally bioavailable dual insulin/IGF-1 receptor tyrosine kinase inhibitor (7). By inhibiting autophosphorylation of these receptors, OSI-906 inhibits their downstream pathways, such as phosphorylation of Akt, ERK1/2, and p70S6K. The half-maximal inhibitory concentrations (IC_{50}) of OSI-906 for the IGF-1 and insulin receptors are

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Abbreviations: ANGPTL8, angiopoietin-like protein 8; BrdU, bromodeoxyuridine; DIO, diet-induced obesity; GKA, glucokinase activator; OGTT, oral glucose tolerance test; PHZ, phloridzin; SGLT, sodium/glucose cotransporter; TGI, tumor growth inhibition.

0.035μM and 0.075μM. OSI-906 has been demonstrated as a promising therapeutic agent for several cancers in animal models (7–11). Insulin and IGF-1 receptor-mediated signaling obviously play a crucial role in the insulin actions regulating the metabolism of glucose and fatty acids. Therefore, unraveling the precise effect of OSI-906 on glycemic control is an important issue for the clinical application of OSI-906, especially in subjects with diabetes.

Decline of the β-cell mass is a hallmark of type 1 and type 2 diabetes, and therapeutic strategies to increase the residual β-cell mass are required for adequate control of diabetes mellitus (12). Insulin and IGF-1 receptors, and their downstream signaling in the β-cells, participate in the maintenance of the β-cell mass and compensatory β-cell mass expansion in response to insulin resistance (3–5, 13, 14). Recently, it was reported that chronic administration of S961, an insulin receptor blocker, induced expression of betatrophin/angiopoietin-like protein 8 (ANGPTL8) in the liver and markedly promoted β-cell proliferation (15). These findings prompted us to investigate whether OSI-906, a dual insulin/IGF-1 receptor blocker, might accelerate or inhibit β-cell proliferation.

In this study, we examined the effects of OSI-906 on the glycemic control in normoglycemic wild-type mice, β-cell–specific glucokinase-haploinsufficient (βGck$^{+/-}$) mice, a model of impaired insulin secretion, and also IRS-2–deficient (IRS-2$^{-/-}$) and diet-induced obesity (DIO) mice, both models of peripheral insulin resistance (12, 13). We also investigated whether OSI-906 can promote β-cell proliferation in mice.

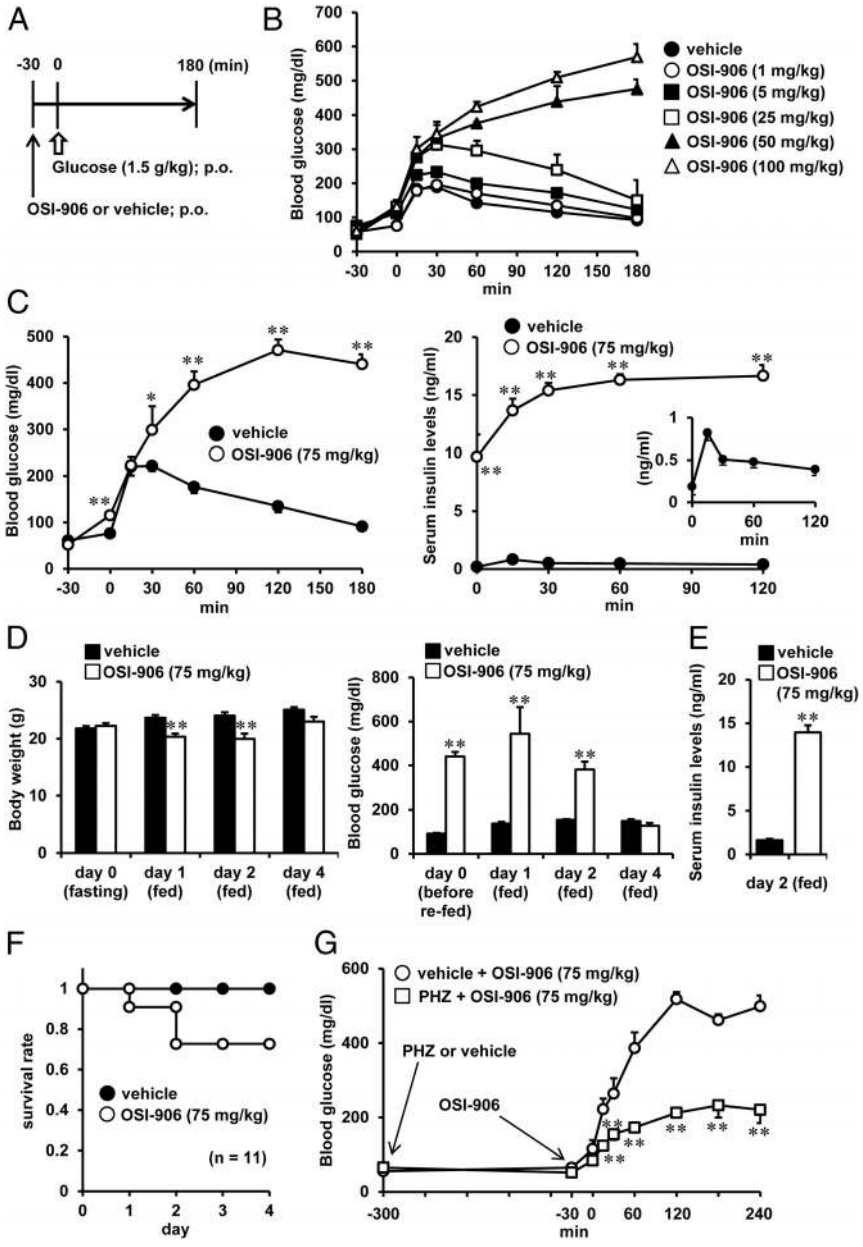

Figure 1. Single administration of a pharmacological dose of OSI-906 (75 mg/kg) worsened the glucose tolerance and survival rate in wild-type mice. The experiments were performed in 8-week-old C57BL/6J mice. A, Experimental protocol. OSI-906 or vehicle (Solutol HS-15) was administered orally 30 minutes before oral glucose loading (1.5 mg/g body weight) for the OGTT. B, Plasma glucose levels during OGTT after administration of indicated doses of OSI-906 (n = 6). C, Plasma glucose levels (n = 11) (left) and serum insulin levels (n = 11) (right) during OGTT after administration of 75 mg/kg OSI-906. *, $P < .05$; **, $P < .01$ vs vehicle. D–F, OSI-906 (75 mg/kg) or vehicle (Solutol HS-15) was administered orally at day 0. The experiments were performed on the indicated day after administration. D, Body weight gain (n = 11) (left) and blood glucose levels (n = 11) (right). **, $P < .01$ vs vehicle. E, Serum insulin levels (n = 11). **, $P < .01$ vs vehicle. F, Survival rate. G, PHZ (800 mg/kg) or vehicle was administered orally 300 minutes before the OGTT. OSI-906 (75 mg/kg) was administered orally 30 minutes before the OGTT. Plasma glucose levels (n = 6). **, $P < .01$ vs vehicle + OSI-906.

Materials and Methods

Animals and animal care

C57BL/6J wild-type mice were obtained from CLEA Japan. We backcrossed βGck$^{+/-}$ mice (12) or IRS-2$^{-/-}$ mice (13) with C57BL/6J mice more than 10 times. DIO mice fed a high-fat diet were also generated as described previously (16). All the experiments were conducted on male littermates. All the animal procedures were performed in accordance with the institutional animal care guidelines and guidelines of the Animal Care Committee of the Yokohama City University. The animal housing

rooms were maintained at a constant room temperature (25°C) and on a 12-hour light (7:00 AM), 12-hour dark (7:00 PM) cycle.

Biochemical parameters and glucose tolerance test

The plasma glucose levels and blood insulin levels were determined using a Glutest Neo Super (Sanwa Chemical Co) and an insulin kit (Morinaga), respectively. All the mice were denied access to food for 20 to 24 hours before the oral glucose tolerance test (OGTT) and then orally loaded with glucose at 1.5 mg/g body weight. For the single administration experiments, 8-week-old mice received oral administration of either vehicle (Solutol HS-15; BASF) or OSI-906 30 minutes before the oral glucose loading (1.5 mg/g). OSI-906 (linstinib) was purchased from Selleck Chemicals and Chemscene. Phloridzin (PHZ) (Sigma) was administered orally 300 minutes before the OGTT (800 mg/kg).

Histological analysis

The mice were ip injected with bromodeoxyuridine (BrdU) (100 mg/kg; Nacalai Tesque, Inc). More than 5 pancreatic tissue sections from each animal were analyzed after fixation and paraffin embedding. The sections were immunostained with antibodies to insulin (Santa Cruz), glucagon (Abcam), Ki67 (DAKO, Japan), BrdU (Sigma), or FoxO1 (Cell signaling technology). Biotinylated secondary antibodies, a Vectastain elite ABC kit, and a diaminobenzidine substrate kit (Vector) were used to examine the sections using bright-field microscopy to determine the β-cell mass, and Alexa Fluor 488-, 555-, and 647-conjugated secondary antibodies (Invitrogen) were used for the fluorescence microscopy. All the images were acquired using a BZ-9000 microscope (Keyence) or FluoView FV1000-D confocal laser scanning microscope (Olympus). The percent area of the pancreatic tissue occupied by the β-cells was calculated using the BIOREVO software (Keyence), as described previously (17). In the BrdU staining experiment, approximately 100 islets were analyzed by BIOREVO software to assess the proportion of immunostained nuclei among the insulin-positive cells in each mouse.

Islet culture

Islets were isolated from the mice as described elsewhere (17). Isolated islets were cultured in RPMI 1640 medium (Wako Pure Chemical Industries) containing 5.6mM glucose supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Islets were treated with 200nM OSI-906 (Selleck Chemicals). All the reagents were added concomitantly to the medium in each experiment.

Real-time PCR

Total RNA was isolated from the pancreatic islets and liver using a ribonuclease-free deoxyribonuclease, RNeasy kit, and QIAshredder (QIAGEN). cDNA was prepared using the High Capacity cDNA reverse transcription kits (Applied Biosystems) and was subjected to quantitative PCR using TaqMan gene expression assays (7900 real-time PCR system; Applied Biosystems) with THUNDERBIRD qPCR Master Mix (Toyobo). All the probes were purchased from Applied Biosystems. Each quantitative reaction was performed in duplicate. Data were normalized according to the β-actin level.

Immunoblotting

For immunoblotting, more than 100 isolated islets were lysed in ice-cold RIPA buffer (Cell Signaling Technology) with complete protease inhibitor cocktail (Roche Diagnostics). After centrifugation, the extracts were subjected to immunoprecipitation with antibodies to the insulin receptor β-subunit (Santa Cruz) or the IGF-1 receptor β-subunit (Cell Signaling Technology), and protein L (Thermo Fisher Scientific). The specimens were subjected to immunoblotting with antibodies to the insulin receptor β-subunit (Santa Cruz), IFG-1 receptor β-subunit (Cell Signaling Technology), or phosphotyrosine (4G10) (Santa Cruz).

Statistical analyses

All the data are expressed as means ± SE and were analyzed using the Student's *t* test or an ANOVA. Differences were considered significant if the *P* value was $< .05$.

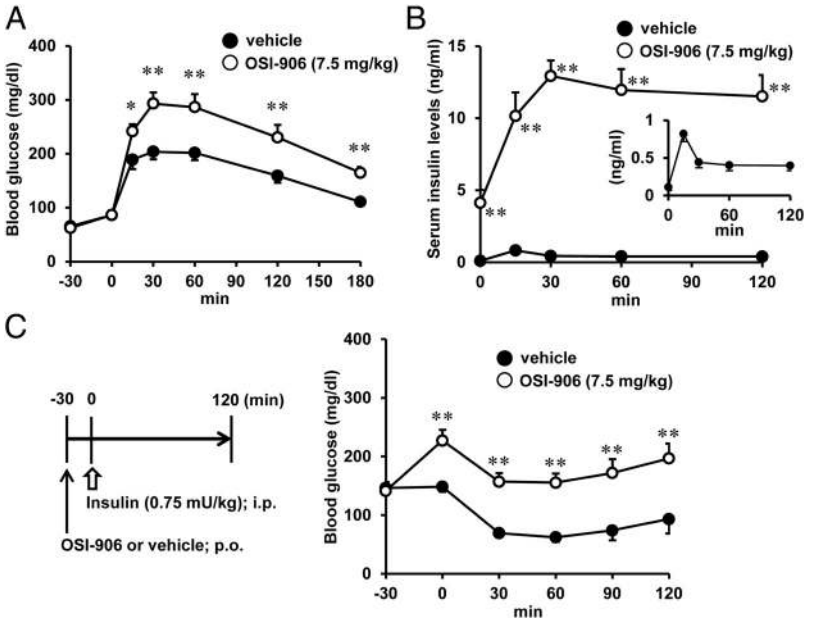

Figure 2. Single administration of a clinical dose of OSI-906 (7.5 mg/kg) worsened the glucose tolerance in wild-type mice. The experiments were performed in 8-week-old C57BL/6J mice. A and B, OGTT after administration of 7.5 mg/kg OSI-906 or vehicle (Solutol HS-15). (n = 7). A, Plasma glucose levels during OGTT. *, $P < .05$; **, $P < .01$ vs vehicle. B, Serum insulin levels during OGTT. **, $P < .01$ vs vehicle. C, Experimental protocol (left) and plasma glucose levels during insulin tolerance test (ITT) (right) (n = 7). OSI-906 (7.5 mg/kg) or vehicle (Solutol HS-15) was administered orally 30 minutes before the ITT. **, $P < .01$ vs vehicle.

Results

Effects of OSI-906 on the glycemic control in wild-type mice

To examine the effect of OSI-906 on the glycemic control, we first administered OSI-906 orally 30 min-

utes before oral glucose loading to euglycemic wild-type C57BL/6J mice (Figure 1A). OSI-906 caused a dose-dependent deterioration of glucose tolerance in the mice (Figure 1B). Dose-dependent tumor growth inhibition (TGI) by OSI-906 has been reported in a xenograft model, with a 75 mg/kg dose causing 100% TGI and 55% regression and a 25 mg/kg dose affording a marginal TGI of 60% and regression rate of 0% (7). Therefore, we next evaluated the effects of OSI-906 at 75 mg/kg as the pharmacological dose. Before elevation of the blood glucose levels, marked elevation of the serum insulin levels was observed after OSI-906 administration (Figure 1C). Reduction of the body weight, hyperglycemia, and hyperinsulinemia were sustained until 2 days after a single administration of OSI-906 at 75 mg/kg OSI-906 (Figure 1, D and E). The drug also reduced the survival rate of the mice (Figure 1F). Because the sodium/glucose cotransporter (SGLT)-1 and SGLT-2 inhibitor PHZ is known to lower blood glucose levels via reducing renal glucose transport, not via insulin receptor signaling, we assessed the effect of this drug on the hyperglycemia elicited by OSI-906. PHZ significantly attenuated the OSI-906–induced elevation of the blood glucose levels (Figure 1G).

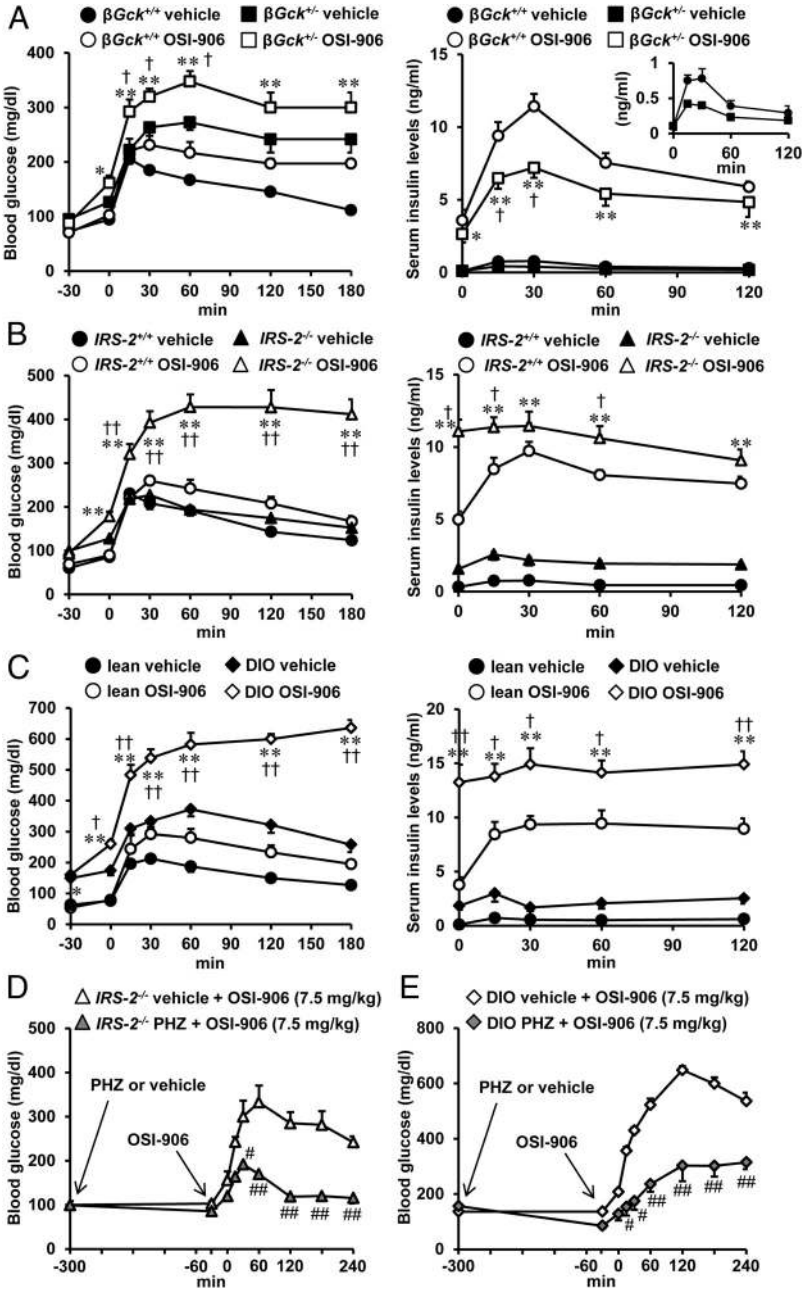

Figure 3. IRS-2–deficient (IRS-$2^{-/-}$) mice and DIO mice exhibited aggravated glucose intolerance after a single administration of OSI-906. A–C, Plasma glucose levels (left) and serum insulin levels (right) during OGTT. OSI-906 (7.5 mg/kg) or vehicle (Solutol HS-15) was administered orally 30 minutes before the OGTT. A, βGck$^{+/-}$ mice or βGck$^{+/+}$ mice (n = 7). B, IRS-$2^{-/-}$ mice or IRS-$2^{+/+}$ mice (n = 7). C, DIO mice or lean mice (n = 10). D and E, Plasma glucose levels in IRS-$2^{-/-}$ mice (n = 5) (D) and in DIO mice (n = 5) (E) during OGTT. PHZ (800 mg/kg) or vehicle (Solutol HS-15) was administered orally 300 minutes before the OGTT as described in Figure 1G. OSI-906 (7.5 mg/kg) was administered orally 30 minutes before OGTT. *, $P < .05$; **, $P < .01$ vs vehicle of each genotype. †, $P < .05$; ††, $P < .01$ vs wild type (βGck$^{+/+}$, IRS-$2^{+/+}$, lean) OSI-906. #, $P < .05$; ##, $P < .01$ vs IRS-$2^{-/-}$ mice vehicle plus OSI-906 or DIO mice vehicle plus OSI-906.

In human clinical trials, OSI-906 has been administered to patients with advanced solid tumors or metastatic colorectal cancers at 150 mg twice daily (300 mg/d) (18). Hence, we examined the effects of OSI-906 administered at the concentration of 7.5 mg/kg as the clinical dose. This dose was sufficient to elicit hyperglycemia, hyperinsulinemia, and insulin resistance in the wild-type mice (Figure 2, A–C). However, all of these changes reversed by 1 day after the administration, and there were no significant changes of the body weight or survival rate between the animals treated with this drug and vehicle-treated mice (data not shown).

OSI-906 worsened glycemic control in the mouse model of insulin resistance

We assessed how the diabetic state might affect the glycemic re-

sponse to OSI-906 in mice. As a model of impaired insulin secretion, we used $\beta Gck^{+/-}$ mice, which lack the brain- and β-cell–specific first exon of the glucokinase gene (12, 19). Although $\beta Gck^{+/-}$ mice showed mild hyperglycemia and impaired insulin secretion in response to glucose under nontreated conditions, the extent of hyperglycemia elicited by 7.5 mg/kg OSI-906 was equal in both genotypes (Figure 3A). In contrast, IRS-$2^{-/-}$ mice exhibited severe glucose intolerance after the administration of OSI-906 at 7.5 mg/kg (Figure 3B). Because insulin resistance is observed in IRS-$2^{-/-}$ mice (13), we next examined DIO mice fed a high-fat diet for 12 weeks. DIO mice also exhibited sustained hyperglycemia and hyperinsulinemia after administration of an identical dose, to the experiments mentioned just before, of OSI-906 (Figure 3C). PHZ significantly improved the OSI-906–induced glucose intolerance in both IRS-$2^{-/-}$ and DIO mice (Figure 3, D and E).

OSI-906 did not affect glucose-induced insulin secretion from isolated islets

We investigated glucose-induced insulin secretion from isolated islets of the wild-type mice in the presence of 200 nM OSI-906. We confirmed that 200 nM OSI-906 reduced the phosphorylation levels of the insulin receptors in the HEK293 cells (Supplemental Figure 1A). FoxO1 was also significantly translocated into the nucleus by 200 nM OSI-906 in the HEK293 cells (Supplemental Figure 1B). The insulin secretion from the islets in response to 2.8 mM, 11.1 mM, and 22.2 mM glucose remained unchanged by treatment with OSI-906 (Figure 4A). OSI-906 also did not modify the expression levels of the insulin genes or glucokinase in the islets in the presence of 2.8 mM or 22.2 mM glucose (Figure 4B).

Chronic administration of OSI-906 increased β-cell proliferation and β-cell mass

Peripheral insulin resistance triggers compensatory β-cell proliferation in mice (16). On the other hand, impaired insulin signaling plays a crucial part in the reactive increment of the β-cell mass (3, 13). OSI-906 is a tyrosine kinase inhibitor of insulin/IGF-1 receptor-mediated signaling. We asked then whether chronic administration of OSI-906 would increase or decrease β-cell proliferation and β-cell mass in mice.

We first gave OSI-906 orally to mice at the dose of 50 mg/kg once daily for 3 days (Supplemental Figure 2A). OSI-906 elicited persistent hyperglycemia and progressive body weight loss (Supplemental Figure 2B). Furthermore, about half of the animals in the OSI-906–treated group died (data not shown). To examine the maximum tolerated dose for repeated oral administration, we next gave OSI-906 orally to the mice at 40 mg/kg once daily for 7 days (Figure 5A). Although this elicited sustained hyperglycemia, no adverse effects on the body weight changes or survival rate were observed (Figure 5, B and C, and data not shown). We validated that this concentration of OSI-906 adequately blunted the phosphorylation of the insulin and IGF-1 receptors in the liver (Supplemental Figure 2C). Concomitantly, the expression levels of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase were significantly increased, and the expression level of glucokinase was significantly decreased in the liver (Supplemental Figure 2D). It is noteworthy that chronic administration of OSI-906 at 40 mg/kg significantly increased the β-cell mass and β-cell proliferation (Figure 5, D and E). The expression levels of betatrophin/*ANGPTL8* in the liver were increased after administration of OSI-906 (Figure 5F).

We also examined the effects of administration of 7.5 mg/kg of OSI-906 once daily for 7 days on the β-cell proliferative activity and β-cell mass (Figure 6A). Chronic administration of OSI-906 at this dose resulted in a slight increase of the blood glucose levels but had no impact on the body weight changes (Figure 6, B and C). This concentration of OSI-906 increased glucose-6-phosphatase expression but had no significant effect on the expression level of phosphoenolpyruvate carboxykinase or glucokinase in the liver (Supplemental Figure 3). Intriguingly, chronic administration of OSI-906 at 7.5 mg/kg also increased the β-cell mass and β-cell proliferative activity (Figure 6, D and E). The betatrophin/*ANGPTL8* expression in the liver increased, although this difference was not statistically significant (Figure 6F).

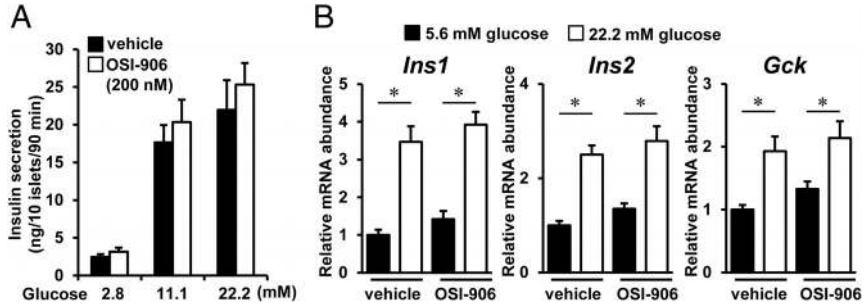

Figure 4. OSI-906 did not affect the insulin secretion from isolated islets. A and B, Islets from C57BL/6J mice (aged 8 weeks) were incubated with 200 nM OSI-906 in the presence of 5.6 mM glucose for 24 hours. A, Insulin secretion by the islets in response to 2.8 mM, 11.1 mM, or 22.2 mM glucose in the presence or absence of 200 nM OSI-906. The results are shown in nanograms insulin per 10 islets per 90 minutes (n = 6). B, mRNA expression levels in the islets (n = 6). *, $P < .05$; **, $P < .01$.

OSI-906 did not inhibit insulin signaling in the β-cells

We also evaluated the inhibition of insulin signaling in the β-cells. Af-

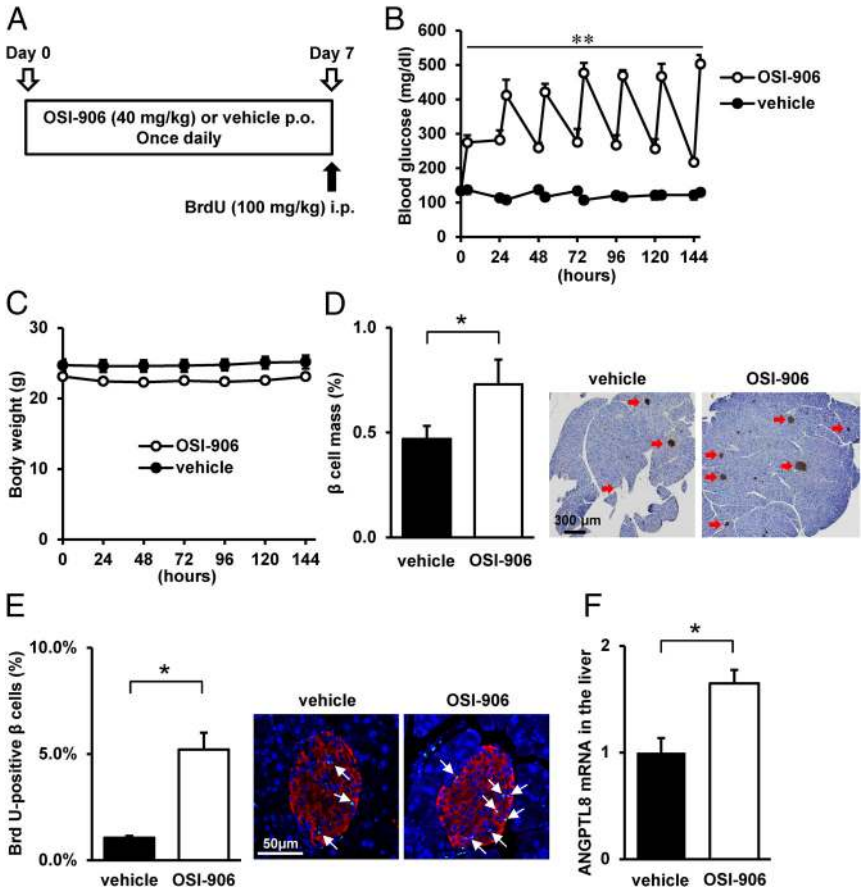

Figure 5. Chronic administration of pharmacological dose of OSI-906 promoted β-cell proliferation. The experiments were performed in 8-week-old C57BL/6J mice (n = 8). A, Experimental protocol. OSI-906 (40 mg/kg) or vehicle was administered orally once daily for 7 days. B, Plasma glucose levels at just before and 4 hours after administration of OSI-906 or vehicle. C, Body weight gain. D, β-cell mass (left), with β-cell area shown as percent area of the entire pancreas, and representative pancreatic sections (right). The red arrows indicate insulin-positive β-cells. E, Number of BrdU-positive β-cells in the islets (at least 30 islets per indicated group, left). Insulin is stained red, nuclei are stained blue (4′,6-diamidino-2-phenylindole [DAPI]), and BrdU-positive nuclei are stained green (right). The white arrows indicate BrdU-positive β-cells. Scale bar, 50 μm. F, mRNA expression levels in the liver (n = 6). *, $P < .05$ vs vehicle.

ter treatment with vehicle or OSI-906 (40 mg/kg) for a week, FoxO1 was mostly localized in the cytoplasm, not in the nuclei, in the insulin-positive β-cells in both the vehicle- and OSI-906–treated mice (Figure 7A). Tyrosine phosphorylation levels of the insulin and IGF-1 receptors in the isolated islets were not significantly downregulated by treatment with 50 mg/kg OSI-906 in vivo (Figure 7B). However, the apparent inhibition of the insulin and IGF-1 receptors were realized in the liver in the same mice (Figure 7B). Glucokinase activation by glucokinase activator (GKA) has been reported to be associated with enhanced expression of *IRS-2*, which results in β-cell proliferation through upregulation of cyclin D1 and D2 (*Ccnd1* and *Ccnd2*) (20–22). Therefore, the effects of OSI-906 on the GKA-induced insulin signal- and the cell cycle-related gene expression in isolated islets were assessed in vitro. In the presence of OSI-906, the induction of *IRS-2*, *PDX1*, cyclin D1, and cyclin D2 gene expression by GKA was preserved, not impaired (Figure 7C). Expression of the cell cycle marker *Ki67* triggered by GKA was also not influenced by OSI-906 treatment (Figure 7C). The phosphorylation levels of the insulin receptor and Akt in the isolated islets were not altered by treatment with OSI-906 in vitro (Supplemental Figure 4B). We could not detect phosphorylation of the IGF-1 receptor in the islets even in the absence of OSI-906 in vitro (Supplemental Figure 4B). These results indicate that inhibition of insulin receptor/IGF-1 receptor by OSI-906 has only feeble activity against insulin signaling in β-cells.

Discussion

Insulin- and IGF-1–mediated signaling via their receptors plays a crucial role in the pathogenesis of metabolic diseases and diabetes, cell proliferation and survival, tumor growth, brain functions, cardiac functions, and longevity (23, 24). OSI-906, a dual inhibitor of the IGF-1 and insulin receptors, has been developed as a promising anticancer agent. In this study, we investigated the effects of OSI-906 on glycemic control, β-cell function, and β-cell proliferation in mice

In previous preclinical studies, OSI-906 was administered to mice at the concentrations of 20, 30, 40, 50, and 75 mg/kg daily (7–9, 11, 25). Administration of OSI-906 at 75 mg/kg to wild-type mice as the pharmacological dose not only elicited severe hyperglycemia, but also resulted in body weight loss and a low survival rate. When OSI-906 was administered to mice at a dose equivalent to that in human clinical trials (7.5 mg/kg or 300 mg/d), the IRS-$2^{-/-}$ mice and DIO mice, which are mouse models of insulin resistance, exhibited more severe hyperglycemia than the wild-type and βGck$^{+/-}$ mice. Hence, insulin resistance might be a predictor of a worsened glycemic response to OSI-906. Hyperinsulinemia compensated OSI-906–induced insulin resistance in the wild-type and βGck$^{+/-}$ mice, because the intrinsic insulin secretory capacity is preserved in both mice. Although βGck$^{+/-}$ mice

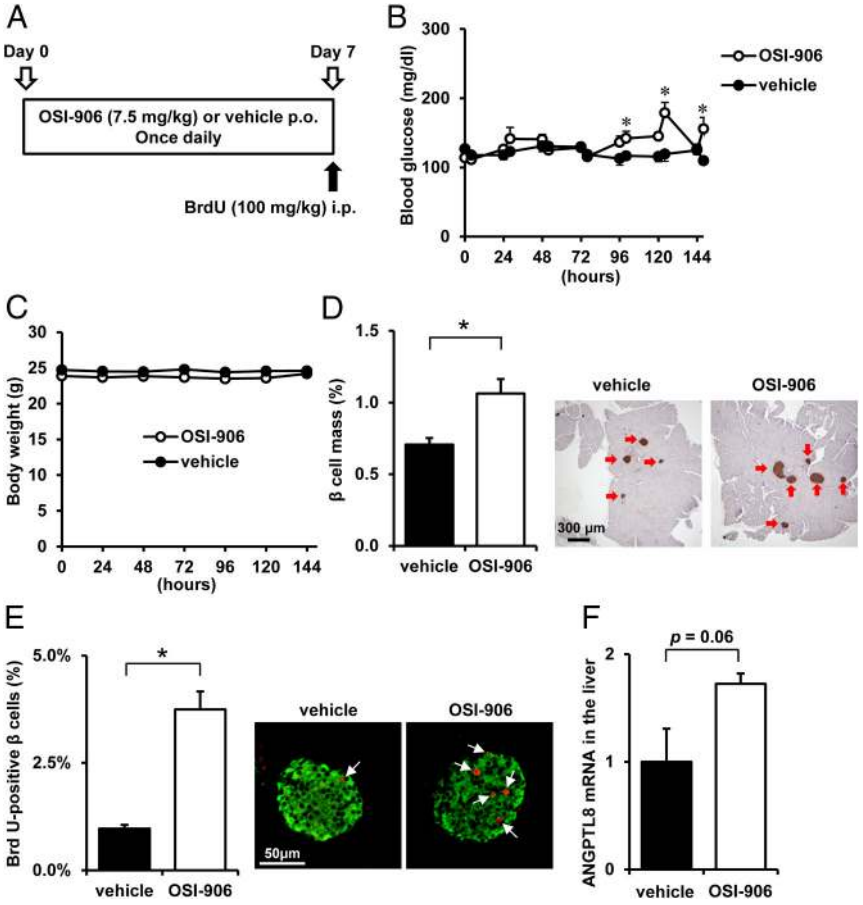

Figure 6. Chronic administration of clinical dose of OSI-906 promoted β-cell proliferation. The experiments were performed in 8-week-old C57BL/6J mice (n = 5). A, Experimental protocol. OSI-906 (7.5 mg/kg) or vehicle was administered orally once daily for 7 days. B, Plasma glucose levels at just before and 4 hours after administration of OSI-906 or vehicle. C, Body weight gain. D, β-cell mass (left), with β-cell area shown as percent area of the entire pancreas, and representative pancreatic sections (right). The red arrows indicate insulin-positive β-cells. E, Number of BrdU-positive β-cells in the islets (at least 30 islets per indicated group, left). Insulin is stained green and BrdU-positive nuclei are stained red (right). The white arrows indicate BrdU-positive β-cells. Scale bar, 50 μm. F, mRNA expression levels in the liver (n = 6). *, $P < .05$ vs vehicle.

exhibit impaired insulin secretion as compared with wild-type mice, a certain level of basal insulin secretion and a compensatory adaptive increase of insulin secretion is conserved in these mice to maintain blood glucose levels. $Ins2^{+/Cys96Tyr}$ Akita mice, which exhibit severely impaired insulin secretion, showed fairly profound hyperglycemia after OSI-906 administration (data not shown). These results suggest that diabetic patients showing severely impaired insulin secretion may develop serious hyperglycemia after treatment with OSI-906. Because PHZ reduced the hyperglycemia significantly even in these mice, use of an SGLT-2 inhibitor might be effective in patients receiving OSI-906.

Because the isolated islets loaded with OSI-906 in vitro showed normal insulin secretion and insulin gene expression, the hyperinsulinemia induced by OSI-906 was not due to its direct actions to on the β-cells. It is possible that hyperglycemia or some other insulin secretagogue was involved in this superfluous insulin secretion to provide stable glycemic control. Tyrosine phosphorylation of the insulin receptor was not affected by OSI-906 treatment in the islets, although dephosphorylation of the insulin receptor was observed in the liver and HEK293 cells. The insulin receptors expressed on the β-cells are presumably exposed to abundant amounts of insulin through autocrine and paracrine mechanisms. Therefore, residual receptors might be hyperactivated by excessive insulin, even after most of the receptors are inhibited by OSI-906. The permeability of drugs through the pancreas depends, in general, on their molecular size and lipid solubility. One possibility is that the distribution of OSI-906 in the pancreatic tissue is insufficient to inhibit the phosphorylation of the insulin/IGF-1 receptors.

Elements of the insulin signaling pathway in the β-cells evidently contribute to β-cell functions, proliferation, and survival (26–29). If OSI-906 elicits pharmacological blockade of the insulin/IGF-1 receptor and downstream insulin signaling in β-cells, it should cause β-cell injury. Our data demonstrated that OSI-906 did not inhibit β-cell proliferation and downstream insulin signaling in the β-cells. Rather, OSI-906 promoted β-cell proliferation and increased the β-cell mass in the mice. As compared with that in a previous study of S961-treated mice (15), the expression level of betatrophin/*ANGPTL8* in the liver and the increment of the β-cell proliferation in the OSI-906–treated mice were mild. The hepatocyte-derived humoral factors contribute to the β-cell proliferative activity in liver-specific insulin receptor knockout (LIRKO) mice (30). Accordingly, blockade of the liver insulin and IGF-1 receptors with OSI-906 may evoke the release of identical systemic factors from hepatocytes. Recent studies have also demonstrated that age-related systemic factors or T cell-produced soluble factors facilitate β-cell proliferation under both physiological and pathological conditions (31, 32). Hence, further study is required to determine the contribution of betatrophin or other humoral factors to the

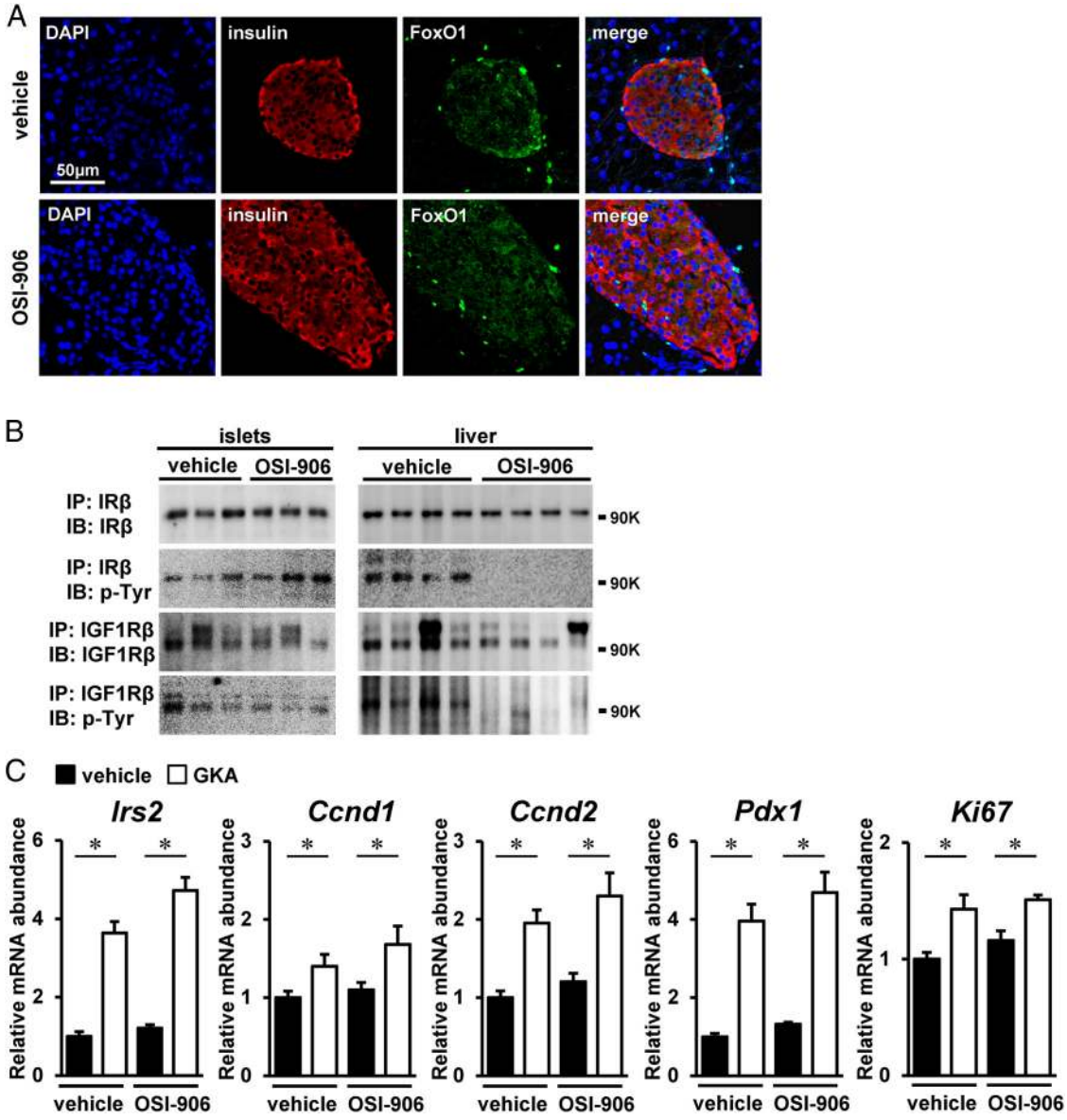

Figure 7. OSI-906 had no effect on the insulin signaling in β-cells. The experiments were performed in 8-week-old C57BL/6J mice (n = 8). A, OSI-906 (40 mg/kg) or vehicle was administered orally once daily for 7 days as described in Figure 5A. Representative pancreatic sections stained with antibodies for insulin (red) and FoxO1 (green) are shown. Scale bar, 50 μm. B, OSI-906 (50 mg/kg) or vehicle was administered orally 3 hours before the analysis. The effect of OSI-906 was confirmed by the detection of hyperglycemia (>300 mg/dL). The total cell extracts from isolated islets were subjected to immunoprecipitation and immunoblotting, as indicated. C, mRNA expression levels in the islets (n = 6). Islets of C57BL/6J mice were incubated with 30μM GKA at 5.6mM glucose for 24 hours, in the presence of 200 nM OSI-906. *, $P < .05$ vs vehicle.

β-cell mass in this model. The proliferative activity of the β-cells was accelerated at both the pharmacological dose (75 mg/kg) and clinical dose (7.5 mg/kg), regardless of the allowable glycemic control and degree of blockade of insulin signaling in the liver in the latter case. These observations suggested that sustained hyperglycemia was not the factor that induced the β-cell replication, and complete loss of insulin signaling was not required for acceleration of the β-cell proliferation in this model. We recently showed that IRS-2$^{-/-}$ mice exhibited a significant increase in β-cell proliferative activity on the sixth day after 60% partial pancreatectomy (33). In this study also, we showed rapid increase of β-cell replication after once-daily administration of OSI-906 for a week. It is possible that the short-term β-cell compensation observed in this model is independent of insulin signaling, such as insulin receptor or IRS-2 signaling.

We recently noted that even if isolated islets were cultured in the presence of OSI-906, GKA satisfactorily increased IRS-2 and regulated the expression of endoplasmic reticulum stress-related genes (20). A recent intriguing review suggested that IRS-2-mediated insulin signaling in the β-cells may not be triggered by activation of the insulin receptor (34). We advocate this concept as being a phys-

iological model for glucose signaling-mediated activation of the insulin signaling pathway in β-cells. β-Cells are reportedly destined to convert to α-cells under severely hyperglycemic conditions (35), whereas the α-cells are converted into β-cells after almost complete elimination of β-cells (36). This transdifferentiation between α-cells and β-cells might be involved in the regulation of the β-cell mass under the chronic hyperglycemic condition elicited by OSI-906. Further study is needed to precisely elucidate the molecular mechanisms by which OSI-906 increases the β-cell mass.

In summary, OSI-906 may worsen glycemic control, especially in patients with insulin resistance, although having no adverse effect on the β-cell mass. We also propose that an SGLT-2 inhibitor may be an ideal candidate as a hypoglycemic drug for patients with hyperglycemia induced by OSI-906. Interestingly, OSI-906 exerts no significant inhibition of the insulin signaling pathway in β-cells. This drug might therefore be useful for elucidating insulin/IGF-1 receptor-mediated signaling in β-cells. Further analyses are needed to clarify the link between OSI-906 and regulation of β-cell function and mass.

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References

1. **Giovannucci E, Harlan DM, Archer MC, et al.** Diabetes and cancer: a consensus report. *Diabetes Care*. 2010;33:1674–1685.
2. **Bowker SL, Majumdar SR, Veugelers P, Johnson JA.** Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin: Response to Farooki and Schneider. *Diabetes Care*. 2006;29:1990–1991.
3. **Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR.** Tissue-specific knockout of the insulin receptor in pancreatic β-cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell*. 1999;96:329–339.
4. **Kulkarni RN, Holzenberger M, Shih DQ, et al.** β-Cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass. *Nat Genet*. 2002;31:111–115.
5. **Ueki K, Okada T, Hu J, et al.** Total insulin and IGF-I resistance in pancreatic β-cells causes overt diabetes. *Nat Genet*. 2006;38:583–588.
6. **Weinstein D, Simon M, Yehezkel E, Laron Z, Werner H.** Insulin analogues display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. *Diabetes Metab Res Rev*. 2009;25:41–49.
7. **Mulvihill MJ, Cooke A, Rosenfeld-Franklin M, et al.** Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. *Future Med Chem*. 2009;1:1153–1171.
8. **Kuhn DJ, Berkova Z, Jones RJ, et al.** Targeting the insulin-like growth factor-1 receptor to overcome bortezomib resistance in preclinical models of multiple myeloma. *Blood*. 2012;120:3260–3270.
9. **Fox EM, Miller TW, Balko JM, et al.** A kinome-wide screen identifies the insulin/IGF-I receptor pathway as a mechanism of escape from hormone dependence in breast cancer. *Cancer Res*. 2011;71:6773–6784.
10. **McKinley ET, Bugaj JE, Zhao P, et al.** 18FDG-PET predicts pharmacodynamic response to OSI-906, a dual IGF-1R/IR inhibitor, in preclinical mouse models of lung cancer. *Clin Cancer Res*. 2011;17:3332–3340.
11. **Kim WY, Prudkin L, Feng L, et al.** Epidermal growth factor receptor and K-Ras mutations and resistance of lung cancer to insulin-like growth factor 1 receptor tyrosine kinase inhibitors. *Cancer*. 2012;118:3993–4003.
12. **Terauchi Y, Sakura H, Yasuda K, et al.** Pancreatic β-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. *J Biol Chem*. 1995;270:30253–30256.
13. **Kubota N, Tobe K, Terauchi Y, et al.** Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory β-cell hyperplasia. *Diabetes*. 2000;49:1880–1889.
14. **Okada T, Liew CW, Hu J, et al.** Insulin receptors in β-cells are critical for islet compensatory growth response to insulin resistance. *Proc Natl Acad Sci U S A*. 2007;104:8977–8982.
15. **Yi P, Park JS, Melton DA.** Betatrophin: a hormone that controls pancreatic β-cell proliferation. *Cell*. 2013;153:747–758.
16. **Terauchi Y, Takamoto I, Kubota N, et al.** Glucokinase and IRS-2 are required for compensatory β-cell hyperplasia in response to high-fat diet-induced insulin resistance. *J Clin Invest*. 2007;117:246–257.
17. **Shirakawa J, Amo K, Ohminami H, et al.** Protective effects of dipeptidyl peptidase-4 (DPP-4) inhibitor against increased β-cell ap-

optosis induced by dietary sucrose and linoleic acid in mice with diabetes. *J Biol Chem*. 2011;286:25467–25476.
18. **King ER, Wong KK.** Insulin-like growth factor: current concepts and new developments in cancer therapy. *Recent Pat Anticancer Drug Discov*. 2012;7:14–30.
19. **Shirakawa J, Tanami R, Togashi Y, et al.** Effects of liraglutide on β-cell-specific glucokinase-deficient neonatal mice. *Endocrinology*. 2012;153:3066–3075.
20. **Shirakawa J, Togashi Y, Sakamoto E, et al.** Glucokinase activation ameliorates ER stress-induced apoptosis in pancreatic β-cells. *Diabetes*. 2013;62:3448–3458.
21. **Salpeter SJ, Klein AM, Huangfu D, Grimsby J, Dor Y.** Glucose and aging control the quiescence period that follows pancreatic β-cell replication. *Development*. 2010;137:3205–3213.
22. **Nakamura A, Togashi Y, Orime K, et al.** Control of β-cell function and proliferation in mice stimulated by small-molecule glucokinase activator under various conditions. *Diabetologia*. 2012;55:1745–1754.
23. **Anisimov VN, Berstein LM, Popovich IG, et al.** Central and peripheral effects of insulin/IGF-1 signaling in aging and cancer: antidiabetic drugs as geroprotectors and anticarcinogens. *Ann N Y Acad Sci*. 2005;1057:220–234.
24. **Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R.** Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev*. 2009;30:586–623.
25. **Zeng X, Zhang H, Oh A, Zhang Y, Yee D.** Enhancement of doxorubicin cytotoxicity of human cancer cells by tyrosine kinase inhibition of insulin receptor and type I IGF receptor. *Breast Cancer Res Treat*. 2012;133:117–126.
26. **Goldfine AB, Kulkarni RN.** Modulation of β-cell function: a translational journey from the bench to the bedside. *Diabetes Obes Metab*. 2012;14(Suppl 3):152–160.
27. **Leibiger IB, Leibiger B, Berggren PO.** Insulin signaling in the pancreatic β-cell. *Annu Rev Nutr*. 2008;28:233–251.
28. **White MF.** Regulating insulin signaling and β-cell function through IRS proteins. *Can J Physiol Pharmacol*. 2006;84:725–737.
29. **Rhodes CJ.** Type 2 diabetes-a matter of β-cell life and death? *Science*. 2005;307:380–384.
30. **El Ouaamari A, Kawamori D, Dirice E, et al.** Liver-derived systemic factors drive β-cell hyperplasia in insulin-resistant states. *Cell Rep*. 2013;3:401–410.
31. **Dirice E, Kahraman S, Jiang W, et al.** Soluble factors secreted by T cells promote β-cell proliferation. *Diabetes*. 2014;63:188–202.
32. **Salpeter SJ, Khalaileh A, Weinberg-Corem N, Ziv O, Glaser B, Dor Y.** Systemic regulation of the age-related decline of pancreatic β-cell replication. *Diabetes*. 2013;62:2843–2848.
33. **Togashi Y, Shirakawa J, Orime K, et al.** β-Cell proliferation after a partial pancreatectomy is independent of IRS-2 in mice. *Endocrinology*. 2014;155:1643–1652.
34. **Rhodes CJ, White MF, Leahy JL, Kahn SE.** Direct autocrine action of insulin on β-cells: does it make physiological sense? *Diabetes*. 2013;62:2157–2163.
35. **Talchai C, Xuan S, Lin HV, Sussel L, Accili D.** Pancreatic β-cell dedifferentiation as a mechanism of diabetic β-cell failure. *Cell*. 2012;150:1223–1234.
36. **Thorel F, Népote V, Avril I, et al.** Conversion of adult pancreatic alpha-cells to β-cells after extreme β-cell loss. *Nature*. 2010;464:1149–1154.