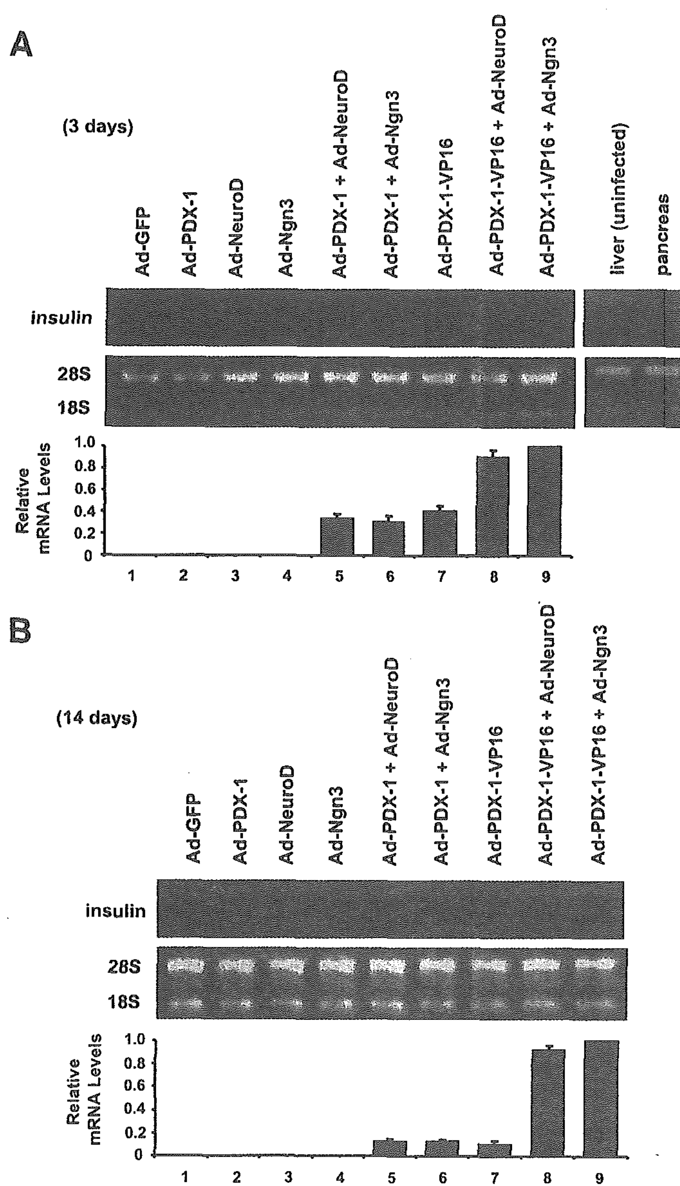

FIG. 6. Quantitative analysis of insulin gene expression induced by adenoviral expression of PDX-1/VP16 in the liver, together with NeuroD or Ngn3. Male C57BL/6 mice were injected with Ad-PDX-1, Ad-NeuroD, Ad-Ngn3, or Ad-GFP (1×10^{10} PFU/ml) into the cervical vein. We then examined insulin mRNA expression levels by Northern blot analysis 3 (A) and 14 (B) days after the injection. Data are means ± SE, with the insulin mRNA levels after treatment with Ad-PDX-1/VP16 plus Ad-Ngn3 being arbitrarily set at 1.0 ($n = 3$). Also shown is insulin mRNA expression in the liver and pancreas without the adenovirus treatment (right panel).

ever, based on various results of insulin expression in this study, it is likely that insulin secretory granules were induced by PDX-1/VP16 overexpression together with NeuroD. Similar secretory granules were observed in the liver after treatment with Ad-PDX-1/VP16 plus Ad-Ngn3 (data not shown). In all the observed cells containing insulin secretory granules, there were several characteristics of hepatocytes, such as big Golgi complex and a large number of mitochondria, indicating that the insulin-producing cells in the liver were hepatocytes, although we cannot deny the possibility that some other cells in the liver also have insulin secretory granules. These results also suggest that hepatocyte-type characteristics are preserved even after insulin expression is induced by the adenovirus infection. Thus PDX-1/VP16 expression together with NeuroD or Ngn3 indeed induces insulin biosynthesis in addition to inducing insulin promoter activity (Fig. 4) and its gene expression (Figs. 5 and 6). Furthermore, to determine whether the insulin produced was secreted into the blood stream, we measured serum immunoreactive insulin levels after a 6-h fast. As shown in Fig. 8, serum insulin levels were significantly increased after treatment with Ad-PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3, although a moderate increase was observed after treatment of Ad-PDX-1 plus Ad-NeuroD, Ad-PDX-1 plus Ad-Ngn3, or Ad-PDX-1/VP16 alone. For reference, we show serum insulin levels after a 6-h fast in untreated nondiabetic mice in Fig. 8. Severe hyperglycemia was not observed after a 6-h fast even in Ad-GFP–treated STZ-induced diabetic mice, and the difference in blood glucose levels after a 6-h fast among the groups was much smaller than the difference in nonfasting blood glucose levels. In particular, blood glucose levels after a 6-h fast in the mice treated with Ad-PDX-1/VP16 plus Ad-NeuroD (or Ad-Ngn3) were similar to those in untreated control mice. However, we cannot exclude the possibility that the

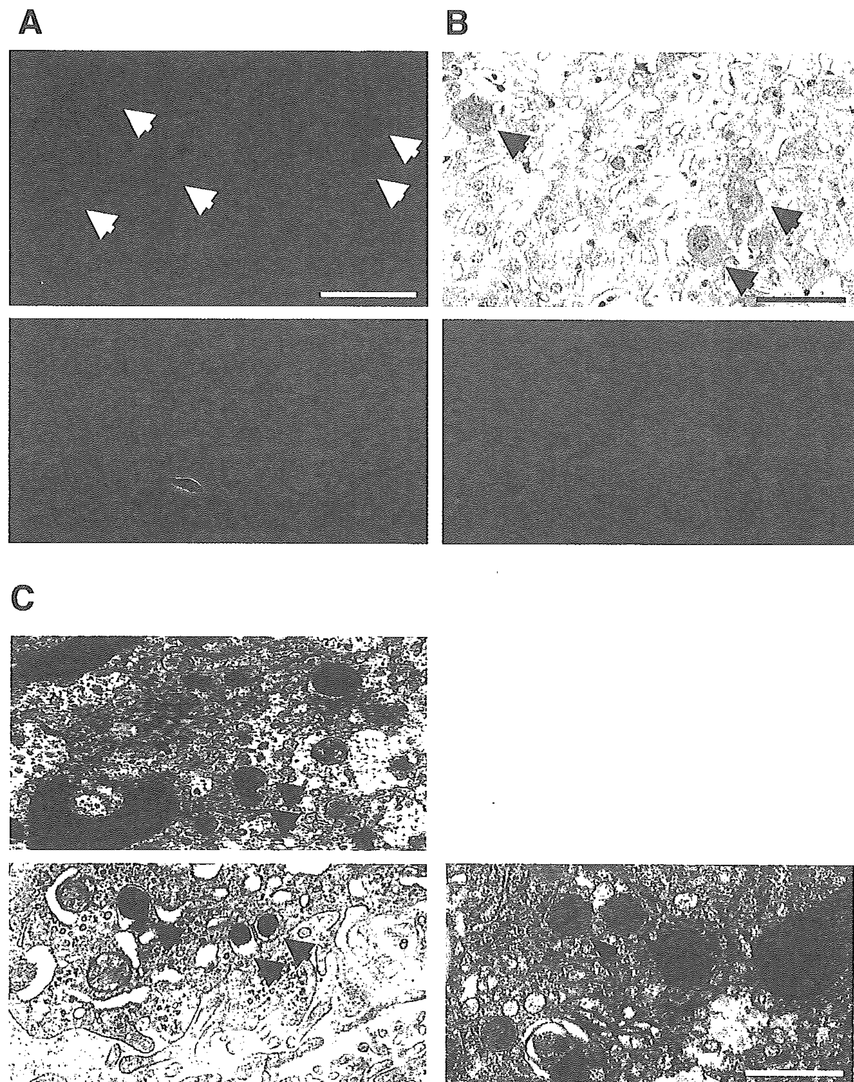

FIG. 7. Adenoviral PDX-1/VP16 expression in the liver, together with NeuroD or Ngn3, induces insulin protein. *A*: To examine insulin protein expression in the liver, we performed immunostaining for insulin after the adenovirus injection. Insulin immunostaining 3 days after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD is shown (*upper panel*). Several insulin-positive cells (*arrows*; *red cells*) were clearly observed in cytoplasm. In contrast, insulin was not detected at all in the control liver (*left lower panel*) or by immunostaining without primary antibody (*right lower panel*). Bar, 20 μm. *B*: To examine insulin processing in the liver, we performed immunostaining for C-peptide after the adenovirus injection. The panel shows C-peptide immunostaining 3 days after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD. Several C-peptide–positive cells (*arrows*; *brown cells*) were clearly observed. Bar, 20 μm. *C*: To further evaluate insulin biosynthesis in the liver, we performed electron microscopy. The panel shows an electron microscopy in the liver 3 days after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD. Several insulin secretory granules (*arrows in left upper and lower panels*) were observed. Peroxisome was observed even in control liver (*arrowheads in right panel*) but was different from insulin secretory granules.

difference in insulin levels among the groups is at least in part due to differences in blood glucose levels.

To examine pancreas-related gene expression caused by ectopic expression of PDX-1, PDX-1/VP16, NeuroD, and Ngn3, we evaluated mRNA expression of various pancreas-related genes by RT-PCR analysis. First, we examined the expression of other β-cell–related genes: islet-type glucokinase, SUR1, and Kir6.2. As shown in Fig. 9*A*, islet-type glucokinase, which is different from hepatic-type glucokinase in the first exon, was detected in the liver after treatment with Ad–PDX-1, Ad–PDX-1 plus Ad-NeuroD or Ad-Ngn3, or Ad–PDX-1/VP16; larger amounts of glucokinase expression were observed after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3. Similarly, large amounts of SUR1 and Kir6.2 mRNA expression were observed after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3, although some expression was detected without these combinations. Also, as shown in Fig. 9*B*, endocrine hormones such as glucagon, somatostatin, and pancreatic polypeptide were detected in the liver after treatment with Ad–PDX-1, and larger amounts of endocrine hormone expression were observed after treatment with Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3. These results suggest that the liver after PDX-1/VP16 overexpression, together with NeuroD or Ngn3, expresses various pancreas-related factors.

To examine whether various pancreatic markers are expressed in the same cells (in insulin-producing cells), we performed immunostaining for various pancreas markers such as glucagon, somatostatin, and pancreatic polypeptide. Although their mRNAs were detected by RT-PCR (Fig. 9*B*), we failed to detect their protein expression by immunostaining, probably because of low (or diffuse) expression of such markers. These results imply that the cells generated by PDX-1/VP16 overexpression, together with NeuroD or Ngn3, are still quite different from real pancreatic endocrine cells. If protein expression levels of such endocrine cell markers were similar to those in real endocrine cells, they should be readily detectable as they are in real endocrine cells. In addition, to examine exocrine cell differentiation caused by ectopic expression of PDX-1, PDX-1/VP16, NeuroD, and Ngn3, we evaluated

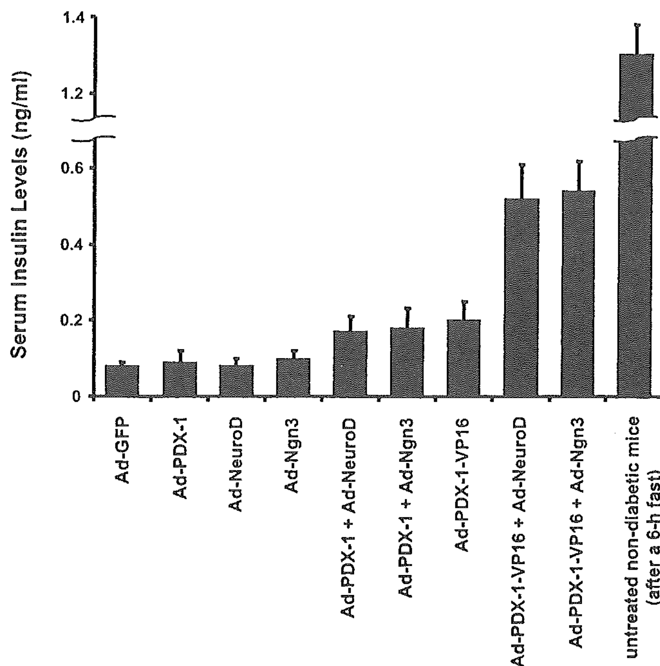

FIG. 8. Adenoviral PDX-1/VP16 expression in the liver, together with NeuroD or Ngn3, substantially increases serum insulin levels. Male C57BL/6 mice were made diabetic with STZ and 1 week later were infected with Ad–PDX-1, Ad–PDX-1/VP16, Ad-NeuroD, Ad-Ngn3, or Ad-GFP. After the adenovirus infection, we determined serum insulin levels. For reference, we show serum insulin levels after a 6-h fast in untreated nondiabetic mice. Data are means ± SE (*n* = 4).

mRNA expression of an exocrine hormone elastase 1. As shown in Fig. 9*B*, elastase 1 mRNA expression was detected after Ad–PDX-1 or Ad–PDX-1/VP16 treatment but was not further increased by the presence of NeuroD or Ngn3. These results suggest that PDX-1 is important for exocrine cell differentiation but that the presence of NeuroD and Ngn3 does not facilitate exocrine cell differentiation.

To examine whether the adenovirus infection and consequent local insulin production influences liver function, 14 days after the adenovirus injection we measured levels of aspartic acid aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels. AST and ALT levels after treatment with Ad-GFP were 40 ± 3 and 29 ± 3 IU/l, respectively, with Ad–PDX-1/VP16 plus Ad-NeuroD were 43 ± 1 and 30 ± 1 IU/l, and with Ad–PDX-1/VP16 plus Ad-Ngn3 were 42 ± 8 and 26 ± 2 IU/l. In addition, 14 days after treatment with Ad-GFP or Ad–PDX-1/VP16 plus Ad-NeuroD or Ngn3, total bilirubin levels in the three groups were within the normal range (<0.1 mg/gl). These results suggest that liver physiology is not influenced by ectopic pancreatic gene expression.

Adenoviral PDX-1/VP16 expression, together with NeuroD or Ngn3, ameliorates glucose tolerance in diabetic model animals more effectively compared with wild-type PDX-1. To examine whether hepatic insulin production induced by PDX-1/VP16 plus NeuroD or Ngn3 is capable of controlling blood glucose levels in diabetic mice, we injected 220 mg/kg STZ into C57BL/6 mice and 1 week later treated the mice with Ad–PDX-1/VP16 with and without Ad-NeuroD or Ad-Ngn3. As shown in Fig. 10*A*, nonfasting blood glucose levels were decreased by PDX-1/VP16 alone, which was a more pronounced change compared with the effects of wild-type PDX-1. As shown in Fig. 10*B*, nonfasting blood glucose levels were further decreased by overexpression of PDX-1/VP16 plus NeuroD or Ngn3. The effects of PDX-1/VP16 plus NeuroD or Ngn3 were more pronounced and persistent compared with those of PDX-1 plus NeuroD or Ngn3. We assume that the capacity of hepatic insulin induced by such transcription factors to lower blood glucose levels in mice with STZ-induced diabetes was due to the induced production and secretion of insulin. Thus, PDX-1/VP16 expression together with NeuroD or Ngn3 markedly induced insulin gene transcription and ameliorated glucose tolerance in diabetic model animals, implying that this combination is useful for replacing the reduced β-cell function found in diabetes.

Furthermore, 3 and 14 days after the adenovirus injection, we performed an intraperitoneal glucose tolerance test. As shown in Fig. 11*A* (3 days) and *B* (14 days), there was a marked difference in glucose tolerance between untreated mice and mice treated with Ad–PDX-1/VP16 plus Ad-NeuroD or Ngn3. In addition, as shown in Fig. 11*C* (3 days) and *D* (14 days), serum insulin levels before glucose challenge in the mice treated with Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3 were much higher than those in mice treated with Ad-GFP. We think that this difference in insulin secretion was due to unregulated production of insulin by Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3. However, there was an additional increase in serum insulin levels in response to a glucose challenge in mice treated with Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3, whereas no such additional increase was observed in mice treated with Ad-GFP. These results suggest that some glucose-mediated regulation of insulin secretion is achieved by Ad–PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3.

DISCUSSION

In this study, we examined the effects of adenovirus-mediated expression of various pancreas-related transcription factors on insulin gene expression and glucose tolerance and found that PDX-1/VP16 overexpression, together with NeuroD or Ngn3, markedly induces insulin

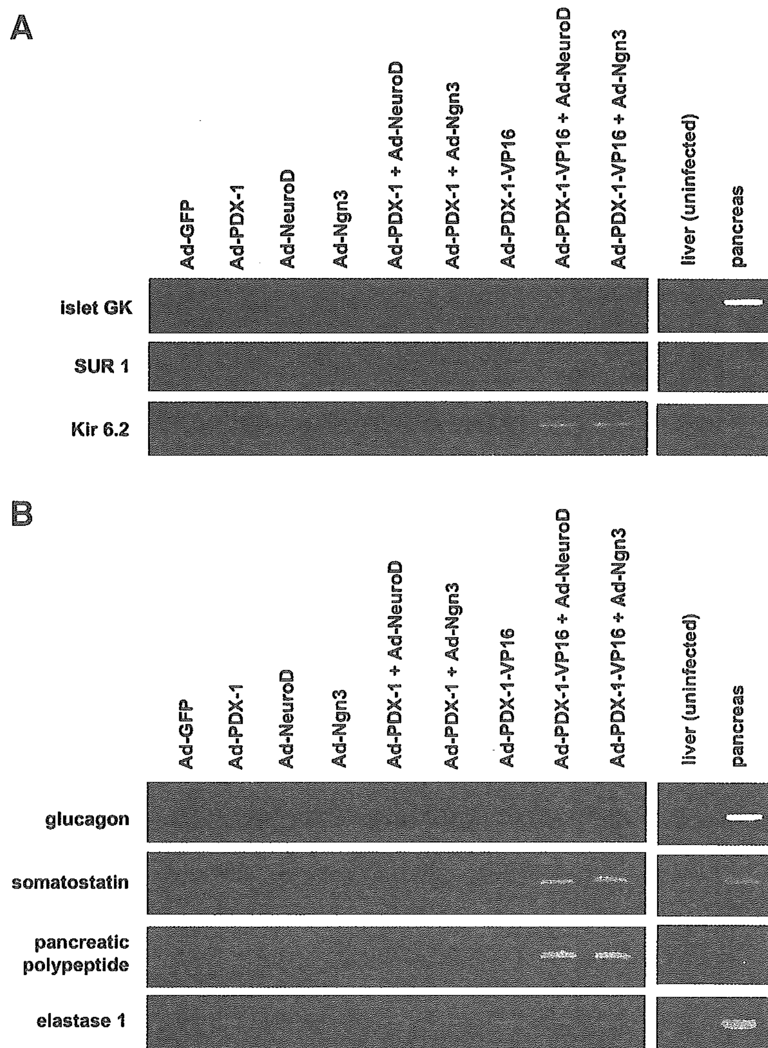

FIG. 9. Adenoviral expression of PDX-1, PDX-1/VP16, NeuroD, and Ngn3 in the liver induces various pancreas-related genes. Male C57BL/6 mice were injected with Ad-PDX-1, Ad-PDX-1/VP16, Ad-NeuroD, Ad-Ngn3, or Ad-GFP (1 × 10^{10} PFU/ml) into the cervical vein. Then, 14 days after the injection, we examined mRNA expression levels of the β-cell–related genes islet-type glucokinase (GK), sulfonylurea receptor 1 (SUR 1), and Kir6.2 (*A*) and the endocrine hormones glucagon, somatostatin, and pancreatic polypeptide (*B*). Also shown is insulin mRNA expression in the liver and pancreas without the adenovirus treatment (*right panel*). Similar results were obtained in three independent experiments.

production in the liver and ameliorates glucose tolerance in diabetic animal models. PDX-1 (1–3) plays a crucial role in pancreas development (4–14), β-cell differentiation (15–26), and the maintenance of normal β-cell function by regulating several β-cell−related genes (14,27–37). Previously it has been shown that PDX-1 functions in concert with other transcription factors (e.g., NeuroD) in regulating the expression of insulin and several other islet-specific genes (39–43) and that VP16 enhances PDX-1 function as a transdifferentiation factor from the liver to the pancreas (72,73). In this study, we examined the effects of PDX-1/VP16 expression on insulin gene expression and glucose tolerance in the absence and presence of other transcription factors such as NeuroD and Ngn3. The marked effects of PDX-1/VP16 expression together with NeuroD or Ngn3 on insulin production and glucose tolerance indicate that the combinations are useful and efficient for replacing the reduced insulin biosynthesis found in diabetes and that PDX-1 requires the recruitment of coordinately functioning transcription factors or cofactors to fully exert its function. PDX-1, NeuroD, and Ngn3 are important molecules for determining the lineage to pancreatic endocrine cells, and thus we thought the combination of such transcription factors would be a useful tool for inducing insulin-producing cells. We would like to emphasize that the combinations of PDX-1/VP16 plus Ngn3 and PDX-1/VP16 plus NeuroD are useful as means to induce insulin-producing cells in non−β-cells and to replace the impaired β-cell function found in diabetes. In our study, many cells in the liver were infected with the adenovirus (Fig. 1*A*), but not all cells expressed substantial amounts of insulin; in fact, ~3 of 100 cells in the liver started expressing substantial amounts of insulin in the liver (Fig. 7*A* and *B*). We assume that because the liver is a very large tissue, the total amounts of insulin biosynthesis in the whole liver were enough to decrease blood glucose levels.

PDX-1/VP16 overexpression, together with NeuroD or Ngn3, substantially induced insulin biosynthesis in the liver (Figs. 5–8) and the various pancreas-related factors that are necessary for β-cell function (Fig. 9). Therefore, we assume that the liver cells started secreting insulin after the adenovirus injection, thus leading to a substantial increase in serum insulin levels (Fig. 8) and marked

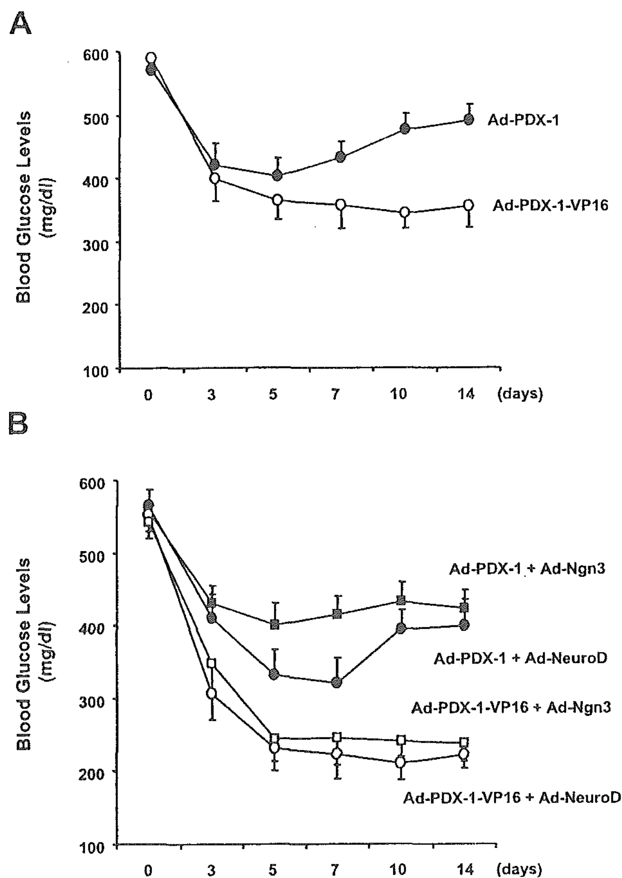

FIG. 10. Adenoviral PDX-1/VP16 expression in the liver, together with NeuroD or Ngn3, markedly decreases blood glucose levels in diabetic animals. Male C57BL/6 mice were made diabetic with STZ and 1 week later were infected with Ad–PDX-1 or Ad–PDX-1/VP16 (*A*) and Ad-PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3 (*B*) (1 × 10^{10} PFU/ml). Nonfasting blood glucose levels were measured with a portable glucose meter after tail snipping. Data are means ± SE (*n* = 6).

improvement of glycemic control (Figs. 10 and 11). In addition, as shown in Fig. 1*B*, the adenovirus is infected only in the liver, when the virus is injected into the cervical vein, suggesting that the improved glycemic control was initiated by a direct effect of adenoviral overexpression of such transcription factors in the liver. However, once the vicious circle of the glucose toxicity is prevented by achieving good glycemic control by some means, the circle then starts moving in the opposite direction: the toxic effects of high glucose are reduced, which results in further improvement of glycemic control. Because the adenoviral overexpression of such transcription factors reduced blood glucose levels (Figs. 10 and 11), it must have reduced glucose toxicity and contributed in part to further improvement of glycemic control.

It has been reported that insulin mRNA expression was detected after Ad–PDX-1 administration alone (16). In that study, the investigators detected insulin mRNA expression by RT-PCR, but not by Northern blot analysis. We also detected insulin mRNA expression by RT-PCR after treatment with Ad–PDX-1 alone (Fig. 2*A* and *B*). As shown in Fig. 6*A* and *B*, however, insulin mRNA was not detected by Northern blot analysis after treatment with Ad–PDX-1 alone (*lane 2*), when film was exposed overnight with an intensifying screen at −80°C. When exposure time was increased up to 3 days, small amounts of insulin mRNA expression were detected by Ad–PDX-1 alone. Because RT-PCR is much more sensitive than Northern blot analysis, we think it is reasonable to hypothesize that insulin mRNA is detected by RT-PCR but not easily detected by Northern blot analysis. In addition, in one study (16), the adenovirus was infected 3 days after STZ administration, whereas we injected the adenovirus 2 weeks after STZ injection. We think that this difference might be important in explaining the difference of the effect of PDX-1 alone on the induction of insulin gene expression. Although not examined in detail in our study, it is likely that 3 days after STZ administration, pancreatic β-cells are not yet completely destroyed. Indeed, in our study, blood glucose levels continued to increase until 1 week after STZ administration. In contrast, it is likely that 2 weeks after STZ administration, β-cells are almost completely destroyed. In addition, it has been reported that although ectopic PDX-1 expression alone is insufficient to induce insulin-producing cells in the liver, hepatic regeneration stimulates the transdifferentiation of the liver into insulin-producing cells; in STZ-induced diabetic mice, adenoviral PDX-1 overexpression plus partial hepatectomy induces substantial amounts of insulin biosynthesis in the liver (26). Those researchers found a significant difference in insulin biosynthesis in the liver with and without partial hepatectomy. Therefore, we assume that in some conditions, PDX-1 alone (without other transcription factors such as NeuroD or Ngn3) is enough to induce some amounts of insulin gene expression in the liver and ameliorate glucose tolerance and that even after β-cells are completely destroyed, the combination of PDX-1/VP16 and NeuroD or Ngn3 can induce substantial amounts of insulin in the liver and markedly ameliorate glucose tolerance.

As shown in Fig. 4, PDX-1 or NeuroD expression induced insulin promoter activity, and cotransfection of these two expression plasmids exerted synergistic effects. Furthermore, the expression of PDX-1/VP16 together with NeuroD or Ngn3 much more markedly induced insulin promoter activity. In addition, as shown in Figs. 5 and 6, insulin gene expression was much more markedly induced by PDX-1/VP16 in the presence of NeuroD or Ngn3. There are several possible explanations as to why the combination of PDX-1/VP16 and NeuroD or Ngn3 is much more active compared with wild-type PDX-1. One possibility is that the transcription partners required for PDX-1 to induce sufficient insulin production are absent in the liver, but that their expression is induced by the combination of PDX-1/VP16 and NeuroD or Ngn3 expression. Indeed, PDX-1 has been shown to require interactions with other proteins such as PBX (76–80), and thus overexpression of unmodified PDX-1 without these protein partners may not be sufficient to induce the production of large amounts of insulin.

In summary, PDX-1/VP16 expression together with NeuroD or Ngn3 markedly induces insulin gene transcription and ameliorates glucose tolerance. This is an approach that warrants further investigation and may turn out to have utility in the treatment of diabetes.

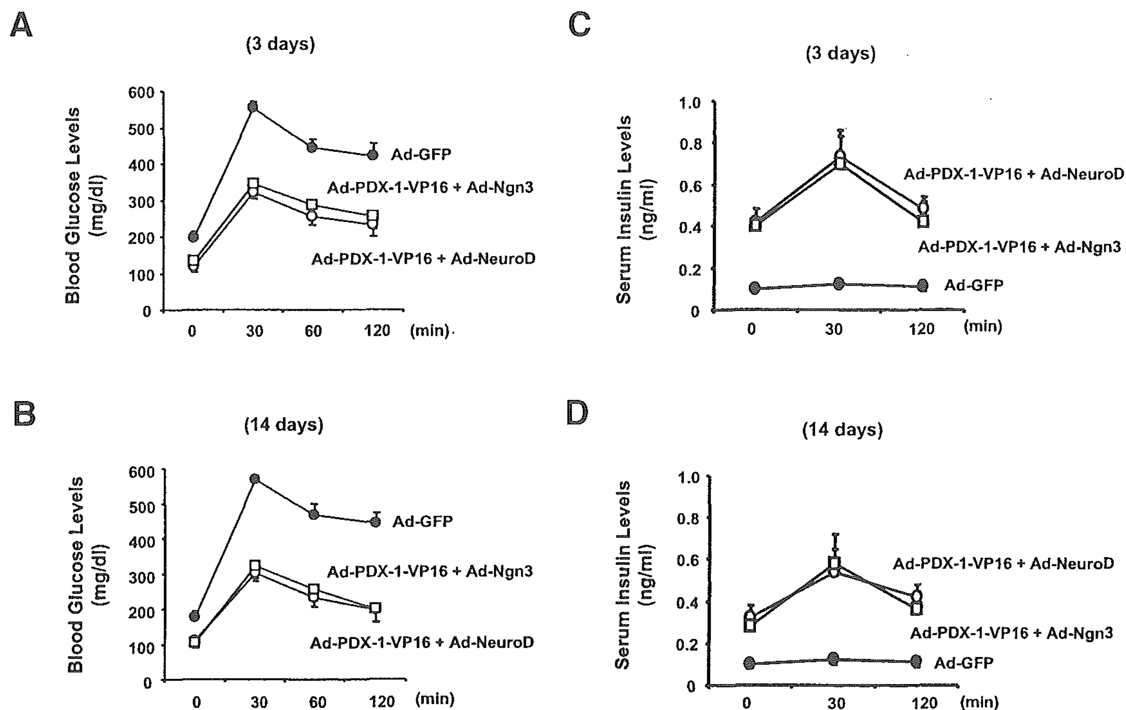

FIG. 11. Adenoviral PDX-1/VP16 expression in the liver, together with NeuroD or Ngn3, markedly ameliorates glucose tolerance in diabetic animals. Male C57BL/6 mice were made diabetic with STZ and 1 week later were infected with Ad-GFP or Ad-PDX-1/VP16 plus Ad-NeuroD or Ad-Ngn3 (1×10^{10} PFU/ml). An intraperitoneal glucose tolerance test was performed 3 and 14 days after the adenovirus infection. Blood glucose levels (*A* and *B*) and serum insulin levels (*C* and *D*) were measured 3 and 14 days after the adenovirus infection. ●, Ad-GFP; ○, Ad-PDX-1/VP16 plus Ad-NeuroD; □, Ad-PDX-1/VP16 plus Ad-Ngn3. Data are means ± SE (n = 4).

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Review

Role of oxidative stress, endoplasmic reticulum stress, and c-Jun N-terminal kinase in pancreatic β-cell dysfunction and insulin resistance

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Abstract

Type 2 diabetes is the most prevalent and serious metabolic disease affecting people all over the world. Pancreatic β-cell dysfunction and insulin resistance are the hallmark of type 2 diabetes. Normal β-cells can compensate for insulin resistance by increasing insulin secretion and/or β-cell mass, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, β-cell function gradually deteriorates and insulin resistance aggravates. Under diabetic conditions, oxidative stress and endoplasmic reticulum stress are induced in various tissues, leading to activation of the c-Jun N-terminal kinase pathway. The activation of c-Jun N-terminal kinase suppresses insulin biosynthesis and interferes with insulin action. Indeed, suppression of c-Jun N-terminal kinase in diabetic mice improves insulin resistance and ameliorates glucose tolerance. Thus, the c-Jun N-terminal kinase pathway plays a central role in pathogenesis of type 2 diabetes and could be a potential target for diabetes therapy.
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Keywords: Diabetes; Oxidative stress; ER stress; The JNK pathway

Abbreviations: Ad, adenovirus; Bip, immunoglobulin binding protein; DN, dominant-negative; eIF2α, α subunit of translation initiation factor 2; ER, endoplasmic reticulum; IRS-1, insulin receptor substrate 1; JIP-1, JNK-interacting protein-1; JNK, c-Jun N-terminal kinase; KDEL, Lys-Asp-Glu-Leu; ORP, oxygen-regulated protein; PERK, pancreatic ER kinase (or PKR-like kinase); PDX-1, pancreatic and duodenal homeobox factor-1; ROS, reactive oxygen species; XBP-1, X-box-binding protein-1

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1. Introduction

The number of patients with type 2 diabetes is markedly increasing worldwide, and nowadays type 2 diabetes is recognized as the most prevalent and serious metabolic disease. In addition, type 2 diabetes is predicted to be an increasing economic and healthcare burden in the near future. Therefore, it is very important to examine a molecular mechanism for pathogenesis of type 2 diabetes and to explore a therapeutic target for diabetes. The development of type 2 diabetes is associated with a combination of pancreatic β-cell dysfunction and insulin resistance. Normal β-cells can compensate for insulin resistance by increasing insulin secretion or β-cell mass, but insufficient compensation leads to the onset of glucose intolerance. Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β-cell function gradually deteriorates and insulin resistance aggravates (Poitout & Robertson, 2002; Weir, Laybutt, Kaneto, Bonner-Weir, & Sharma, 2001). This process is called "glucotoxicity" (Fig. 1). Similar to the paradoxically deleterious effects of chronic hyperglycemia, free fatty acids, which are essential fuels in the normal state, become toxic when they are chronically present in excessive levels (Unger, 1995). This process is called "lipotoxicity" (Fig. 1). Under such conditions, oxidative stress and endoplasmic reticulum (ER) stress are provoked and the c-Jun N-terminal kinase (JNK) pathway is activated in various tissues. Here we show a role of oxidative stress, ER stress, and the JNK pathway in pancreatic β-cell dysfunction and insulin resistance; the JNK activation suppresses insulin biosynthesis and interferes with insulin action and, in reverse, suppression of the JNK pathway in diabetic mice improves insulin resistance and ameliorates glucose tolerance.

2. Role of oxidative stress, ER stress, and the JNK pathway in pancreatic β-cell dysfunction

2.1. Oxidative stress and pancreatic β-cell dysfunction

Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β-cell function gradually deteriorates (Jonas et al., 1999; Moran et al., 1997; Poitout and Robertson, 2002; Weir et al., 2001). This process is called "glucotoxicity" (Fig. 1). The mechanism for glucotoxicity is, at least in part, mediated by overloads of reactive oxygen species. Similar to the paradoxically deleterious effects of chronic hyperglycemia, free fatty acids, which are essential fuels in the normal state, become toxic when they are chronically present in excessive levels (Unger, 1995). This process is called "lipotoxicity" (Fig. 1). Prolonged exposure of β-cells to free fatty acids increases basal insulin release but inhibits glucose-induced insulin secretion (Bollheimer,

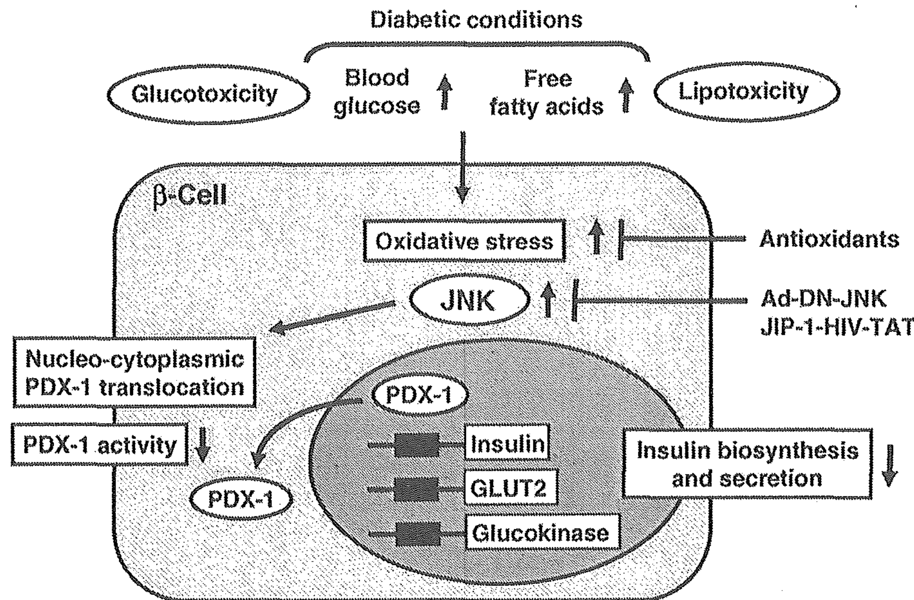

Fig. 1. Involvement of oxidative stress and the JNK pathway in pancreatic β-cell dysfunction. Increase of oxidative stress and activation of the JNK pathway suppresses insulin biosynthesis and secretion through nucleo-cytoplasmic PDX-1 translocation.

Skelly, Chester, McGarry, & Rhodes, 1998; Gremlich, Bonny, Waeber, & Thorens, 1997; Jacqueminet, Briaud, Rouault, Reach, & Poitout, 2000). In addition, free fatty acids inhibit insulin gene expression and finally induce apoptosis in β-cells (Shimabukuro, Zhou, Levi, & Unger, 1998).

Physiological levels of reactive oxygen species are important to maintain various cell function, but overloads of reactive oxygen species that exceed the capacity of the antioxidant system induce oxidative stress. Oxidative stress is involved in various diseases such as inflammation, carcinogenesis, and atherosclerosis. Also, it has been shown recently that oxidative stress plays a role in the progression of diabetes. Under diabetic conditions, oxidative stress is provoked in various tissues including pancreatic β-cells (Baynes & Thorpe, 1999; Brownlee, 2001; Gorogawa et al., 2002; Ihara et al., 1999; Nishikawa et al., 2000). Because expression levels of antioxidant enzymes such as catalase, and glutathione peroxidase were very low in β-cells compared to other tissues (Lenzen, Drinkgern, & Tiedge, 1996; Tiedge, Lortz, Drinkgern, & Lenzen, 1997), β-cells are thought as a target of oxidative stress-mediated tissue damage (Evans, Goldfine, Maddux, & Grodsky, 2002; Maechler, Jornot, & Wollheim, 1999; Robertson, Harmon, Tran, Tanaka, & Takahashi, 2003; Tanaka, Gleason, Tran, Harmon, & Robertson, 1999; Tanaka, Tran, Harmon, & Robertson, 2002). Thus, it is likely that oxidative stress is involved in β-cell deterioration in type 2 diabetes. There are several sources of reactive oxygen species (ROS) productions in cells: the non-enzymatic glycosylation reaction (Kaneto et al., 1996; Matsuoka et al., 1997), the electron transport chain in mitochondria (Nishikawa et al., 2000; Sakai et al., 2003), and the hexosamine pathway (Kaneto et al., 2001). It was previously shown that oxidative stress suppresses the insulin gene transcription in β-cells; when β-cell-derived HIT cells or isolated rat islets were exposed to oxidative stress, insulin mRNA expression was suppressed (Kaneto et al., 2001; Matsuoka et al., 1997). As a possible cause of the reduction in the insulin gene promoter activity by oxidative stress, it was shown that the DNA-binding activity of pancreatic and duodenal homeobox factor-1 (PDX-1) is rather sensitive to oxidative stress; when HIT cells or isolated rat islets were exposed to oxidative stress, PDX-1 binding to the insulin gene was markedly reduced (Kaneto et al., 2001; Matsuoka et al., 1997). PDX-1, also known as IDX-1/STF-1/IPF1 (Leonard et al., 1993; Miller, McGehee, & Habener, 1994; Ohlsson, Karlsson, & Edlund, 1993), is a member of the homeodomain-containing transcription factor family, and plays a cru-

cial role in pancreas development and differentiation (Cao, Tang, Horb, Li, & Yang, 2004; Dutta, Bonner-Weir, Montminy, & Wright, 1998; Ferber et al., 2000; Holland, Hale, Kagami, Hammer, & MacDonald, 2002; Horb, Shen, Tosh, & Slack, 2003; Jonsson, Carlsson, Edlund, & Edlund, 1994; Kaneto et al., 2005; Miyatsuka et al., 2003; Stoffers, Zinkin, Stanojevic, Clarke, & Habener, 1997; Taniguchi et al., 2003), and in maintaining normal β-cell function by regulating multiple important β-cell genes, including insulin, GLUT2, and glucokinase (Ahlgren, Jonsson, Jonsson, Simu, & Edlund, 1998; Brissova et al., 2002; Chakrabarti, James, & Mirmira, 2002; Kulkarni et al., 2004; Peers, Leonard, Sharma, Teitelman, & Montminy, 1994; Petersen,Serup, Leonard, Michelsen, & Madsen, 1994; Waeber, Thompson, Nicod, & Bonny, 1996; Wang et al., 2001; Watada et al., 1996). Taken together, oxidative stress-induced under diabetic conditions suppresses insulin biosynthesis, accompanied by reduction of PDX-1 DNA-binding activity (Fig. 1). Next, to evaluate the potential usefulness of antioxidants in treatment for type 2 diabetes, obese diabetic C57BL/KsJ-db/db mice were treated with antioxidants (*N*-acetyl-L-cysteine plus Vitamins C and E). The antioxidant treatment retained glucose-stimulated insulin secretion and moderately decreased blood glucose levels. The β-cell mass was significantly larger in the mice treated with the antioxidants. The amounts of insulin content and insulin mRNA were also preserved by the antioxidant treatment (Kaneto et al., 1999a, 1999b). Similar effects were observed with Zucker diabetic fatty (ZDF) rats, another model animals for type 2 diabetes (Tanaka et al., 1999). These data indicate that antioxidant treatment can protect β-cells against glucose toxicity. In addition, treatment with probucol, an antioxidant widely used as an anti-hyperlipidemic agent, preserved β-cell mass, the insulin content, and glucose-stimulated insulin secretion, leading to improvement of glucose tolerance (Gorogawa et al., 2002). These data suggest potential usefulness of antioxidants for diabetes and provides further support for the implication of oxidative stress in β-cell glucose toxicity found in diabetes (Fig. 1).

2.2. *ER stress and pancreatic β-cell dysfunction*

The endoplasmic reticulum (ER) is an organelle which synthesizes various secretory and membrane proteins. These proteins are correctly folded and assembled by chaperones in the ER. During stressful conditions such as upon an increase in the misfolded protein level, the chaperons become overloaded and the ER fails to fold and export newly synthesized proteins, leading to ER stress (Aridor & Balch, 1999; Harding, Zhang, & Ron, 1999; Ron, 2002; Tirasophon, Welihinda, & Kaufman, 1998; Wang et al., 1998). Once ER stress is provoked in the cells, various pathway is activated (Fig. 2). It was previously reported that ER stress is involved in pancreatic β-cell apoptosis (Harding & Ron, 2002; Harding et al., 2001; Inoue et al., 1998; Oyadomari et al., 2001, 2002). The Akita mice harbor a spontaneous mutation which causes early-onset non-obese diabetes. Diabetes is caused by a missense mutation, insulin 2 C96Y, which replaces a highly conserved cysteine with tyrosine. This mutation precludes formation of one of the two disulfide bonds which are normally present in proinsulin-2. Since the mutant proinsulin is retained in the ER, the mice are unable to produce enough amounts of insulin and becomes diabetic. Also, it was shown that ER stress is directly involved in the decrease of β-cell mass; when the Akita mutation was introduced into CHOP-KO mice, β-cell mass in the mice was preserved and the onset of hyperglycemia was delayed (Oyadomari et al., 2002). Therefore, it is likely that once ER stress is increased by some stimuli, ER stress triggers apoptosis in β-cells and thus ER stress is involved in progression of β-cell dysfunction and/or death found in diabetes. In addition, it is known that protein synthesis is rapidly repressed in cells experiencing ER stress. The pancreatic ER kinase (or PKR-like kinase) (PERK) is an ER transmembrane protein kinase that phosphorylates the α subunit of translation initiation factor 2 (eIF2α) in response to ER stress, leading to reduction of protein translation (Fig. 2). Indeed, it was shown that decreased protein synthesis observed in ER stressed cultured wild type cells is completely absent from similarly treated PERK-KO mice (Harding, Zhang, Bertolotti, Zeng, & Ron, 2000). Furthermore, the mutant cells were markedly hypersensitive to treatment with agents which induce ER stress such as tunicamycin and thapsigargin. The mice are born with nearly normal pancreatic islets, but over the first few weeks of life, they experience a progressive destruction of β-cells (Harding et al., 2001). These results further indicate an involvement of ER stress in progression of β-cell dysfunction.

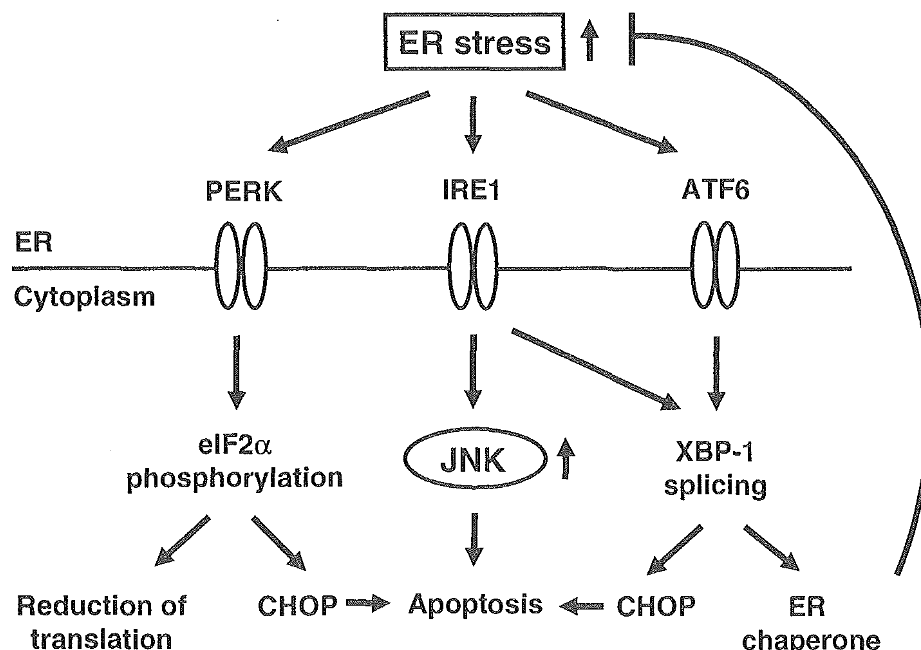

Fig. 2. ER stress signaling. Once ER stress is induced in the cells, various pathways are activated: eIF2α phosphorylation, JNK activation, and XBP-1 splicing.

2.3. *The JNK pathway and pancreatic β-cell dysfunction*

When cells are exposed to various stress, several selected proteins are expressed and respond to such stress. Indeed, several signal transduction pathways including c-Jun N-terminal kinase (JNK) (also known as stress-activated protein kinase (SAPK)) (Chang & Karin, 2001; Davis, 2000; Derijard et al., 1994; Hibi, Lin, & Karin, 1993), p38 mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) are known to be activated by oxidative stress, ER stress, and/or high glucose in several cell types. Recently it was shown that the JNK pathway is activated by oxidative stress in pancreatic β-cells and that activation of the JNK pathway is involved in reduction of insulin gene expression by oxidative stress and that suppression of the JNK pathway can protect β-cells from oxidative stress (Kaneto et al., 2002). When isolated rat islets were exposed to oxidative stress, the JNK pathway was activated, preceding the decrease of insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type JNK1 (DN-JNK) protected insulin gene expression and secretion from oxidative stress. Moreover, wild type JNK1 (WT-JNK) overexpression suppressed both insulin gene expression and secretion (Kaneto et al., 2002). These results were correlated with changes in the binding of the important transcription factor PDX-1 to the insulin promoter; adenoviral overexpression of DN-JNK preserved PDX-1 DNA-binding activity in the face of oxidative stress, while WT-JNK overexpression decreased PDX-1 DNA-binding activity (Kaneto et al., 2002). Therefore, it is likely that activation of JNK pathway leads to decreased PDX-1 activity and subsequent suppression of insulin gene transcription in the diabetic state (Fig. 1). Furthermore, as a potential mechanism for JNK-mediated PDX-1 inactivation, it was recently reported that PDX-1 was translocated from the nuclei to the cytoplasm in response to oxidative stress. When oxidative stress was charged upon β-cell-derived HIT cells, PDX-1 moved from the nuclei to the cytoplasm (Kawamori et al., 2003). Addition of DN-JNK inhibited the oxidative stress-induced PDX-1 translocation, suggesting an essential role of JNK in mediating the phenomenon. In addition, leptomycin B, a specific inhibitor of the classical, leucine-rich nuclear export signal (NES), inhibited nucleo-cytoplasmic transloca-

tion of PDX-1 induced by oxidative stress (Fig. 1) (Kawamori et al., 2003). Also, it was reported that DN-JNK can protect β-cells from some of the toxic effects of hyperglycemia during this transplant period, providing new insights into the mechanism through which oxidative stress suppresses insulin gene transcription in β-cells. It was reported that β-cell destruction by cytokines such as interleukin-1 β (IL-1β) (Corbett, Wang, Sweetland, Lancaster, & McDaniel, 1992; Eizirik et al., 1994; Kaneto et al., 1995) can be prevented by inhibition of the JNK pathway (Ammendrup et al., 2000; Bonny et al., 2000, 2001; Mandrup-Poulsen, 2001), implying that JNK plays a role in autoimmune β-cell destruction found in early stage of type 1 diabetes. Taken together, it is likely that the JNK pathway is involved in deterioration of β-cell function in both type 2 diabetes and the early stage of type 1 diabetes (Fig. 1).

3. Role of oxidative stress, ER stress, and the JNK pathway in insulin resistance

3.1. *Oxidative stress and insulin resistance*

The hallmark of the disease is insulin resistance as well as pancreatic β-cell dysfunction. Under diabetic conditions, various insulin target tissues such as liver, muscle, and fat become resistant to insulin. The pathophysiology of insulin resistance involves a complex network of insulin signaling pathways. After insulin binds to insulin receptor on cell surface, insulin receptor and its substrates are phosphorylated, which leads to activation of various insulin signaling pathways. It has been shown that oxidative stress is involved in progression of insulin resistance as well as pancreatic β-cell dysfunction (Evans et al., 2002). It was previously reported that oxidative stress disrupted insulin-induced cellular redistribution of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI 3-K) and thus impaired insulin-induced GLUT4 translocation in 3T3-L1 adipocyte (Tirosh, Potashnik, Bashan, & Rudich, 1999). Also, it was also reported that treatment with antioxidants (*N*-acetyl-L-cysteine and taurine) prevented hyperglycemia-induced insulin resistance in vivo (Haber et al., 2003). Furthermore, in patients with type 2 diabetes, both acute and chronic administration of α-lipoic acid, an antioxidant, improves insulin resistance as measured by both the euglycemic hyperinsulinemic clamp and the Bergman minimal model (Jacob et al., 1999; Konrad et al., 1999). These data indicate that oxidative stress is involved in progression of insulin resistance.

3.2. *ER stress and insulin resistance*

Under diabetic conditions, ER stress is increased in various tissues. Expression levels of immunoglobulin binding protein (Bip) and Lys-Asp-Glu-Leu (KDEL), both of which are ER stress markers, were much higher in the liver in the obese diabetic mice compared to non-diabetic C57BL6 mice (Nakatani et al., 2005). It was also reported about the expression of several ER stress markers in dietary (high-fat diet-induced) and genetic (ob/ob) models of obesity. The pancreatic ER kinase (or PKR-like kinase) (PERK) is an ER transmembrane protein kinase that phosphorylates the α subunit of translation initiation factor 2 (eIF2α) in response to ER stress, leading to reduction of protein translation (Fig. 2). Therefore, phosphorylation status of PERK and eIF2α is a key indicator of the presence of ER stress (Shi, Taylor, Tan, & Sonenberg, 2003; Shi et al., 1998). PERK and eIF2α phosphorylation were increased in the liver of obese mice compared with lean control. It is known that the activity of c-Jun N-terminal kinase (JNK) is increased by ER stress (Fig. 2) (Urano et al., 2000). Indeed, total JNK activity was also dramatically elevated in the obese mice (Ozcan et al., 2004). It was also reported that when Fao liver cells were treated with tunicamycin or thapsigargin, both of which are ER stress inducers, insulin-stimulated tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) was significantly decreased. IRS-1 is a substrate for insulin receptor tyrosine kinase, and serine phosphorylation of IRS-1, particularly mediated by JNK, reduces insulin receptor signaling (Aguirre, Davis, & White, 2000). Indeed, pretreatment of Fao cells with tunicamycin produced a significant increase in serine phosphorylation of IRS-1 (Ozcan et al., 2004). Furthermore, inhibition of JNK activity with the synthetic inhibitor, SP600125, reversed the ER stress-induced serine phosphorylation of IRS-1. These results indicate that ER stress promotes a JNK-dependent serine phosphorylation of IRS-1, which in turn inhibits insulin signaling (Fig. 3) (Ozcan et al., 2004).

Furthermore, it was reported that mice deficient in X-box-binding protein-1 (XBP-1), a transcription

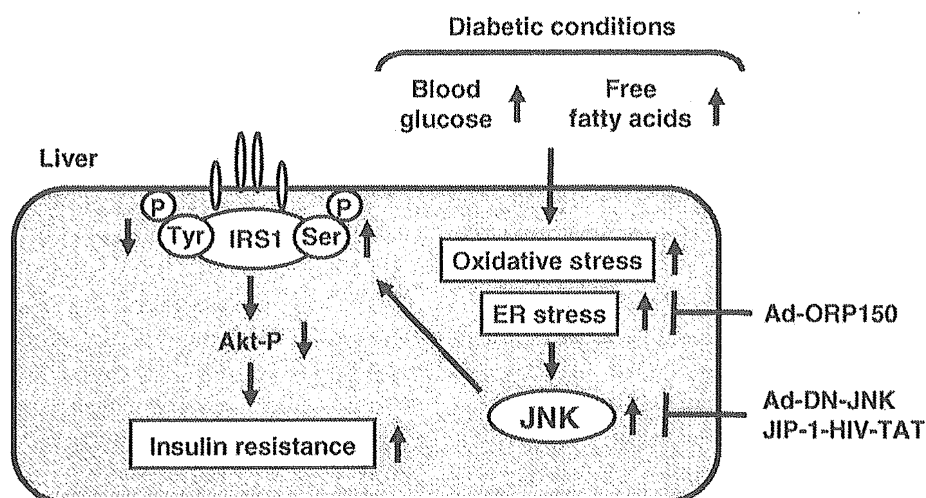

Fig. 3. Involvement of ER stress and the JNK pathway in insulin resistance. Increase of ER stress and activation of the JNK pathway are involved in insulin resistance found in type 2 diabetes.

factor that modulates the ER stress response, develop insulin resistance (Ozcan et al., 2004). The spliced form of XBP-1 is a key factor in ER stress through transcriptional regulation of various genes, including molecular chaperones (Fig. 2). Since complete XBP-1 deficiency results in embryonic lethality, BALB/c-XBP-$1^{+/-}$ mice with a null mutation in one XBP-1 allele were used in order to investigate the role of XBP-1 in insulin resistance and diabetes in vivo (Ozcan et al., 2004). XBP-$1^{+/-}$ mice treated with high-fat diet developed continuous and progressive hyperinsulinemia. Blood glucose levels were increased in the XBP-$1^{+/-}$ mice treated with high-fat diet. In insulin tolerance test, the hypoglycemic response to insulin was also significantly lower in XBP-$1^{+/-}$ mice compared with XBP-$1^{+/+}$ littermates (Ozcan et al., 2004). Increase of PERK phosphorylation was observed in the liver of obese XBP-$1^{+/-}$ mice treated with high-fat diet. There was also a significant increase in the JNK activity in the XBP-$1^{+/-}$ mice. Consistently, Ser^{307} phosphorylation of IRS-1 was increased in XBP-$1^{+/-}$ mice compared with wild type control. There was no detectable difference in any of the insulin receptor signaling components in the liver and adipose tissues between genotypes taking regular diet. However, after treatment with high-fat diet, major components of insulin receptor signaling in the liver, including IRS-1 tyrosine- and Akt serine-phosphorylation, were all decreased in XBP-$1^{+/-}$ mice compared with wild type control. A similar suppression of insulin receptor signaling was also observed in the adipose tissues of the XBP-$1^{+/-}$ mice (Ozcan et al., 2004). Taken together, induction of ER stress leads to suppression of insulin receptor signaling via IRE-1α-dependent activation of the JNK pathway. Furthermore, deletion of an XBP-1 allele in mice led to systemic insulin resistance and type 2 diabetes. Therefore, it is likely that ER stress is involved in progression of insulin resistance and thus could be a potential therapeutic target for diabetes (Fig. 3).

3.3. ER stress as a potential therapeutic target for insulin resistance

Oxygen-regulated protein 150 (ORP150) (Kuwabara et al., 1996), a molecular chaperone found in the ER, has been shown to protect cells from ER stress. To examine a role of ER stress in insulin resistance in vivo, ORP150 expressing adenovirus (Ad-ORP) were delivered to 8-week-old C57BL/KsJ-db/db obese diabetic mice from the cervical vein. An increase in ORP150 expression in the liver was confirmed, but not in other tissues such as muscle and adipose tissue, was confirmed upon adenovirus injection. In addition, expression levels of KDEL and Bip in Ad-ORP-treated mice were lower compared to those in Ad-GFP treated db/db mice, indicating that ORP150 is actually acting to decrease ER stress in the liver. When C57BL/KsJ-db/db mice were treated with

Ad-ORP, glucose tolerance was markedly ameliorated. In intraperitoneal insulin tolerance test (IPITT), the hypoglycemic response to insulin was larger in Ad-ORP-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. Also, in the euglycemic hyperinsulinemic clamp test, the glucose infusion rates (GIR) of Ad-ORP-treated mice were significantly higher compared to Ad-GFP-treated mice, indicating that ORP150 overexpression in the liver reduces insulin resistance and thus ameliorates glucose tolerance in C57BL/KsJ-db/db mice. Endogenous hepatic glucose production (HGP) was significantly lower in Ad-ORP-treated mice compared to Ad-GFP-treated mice (Fig. 3) (Nakatani et al., 2005). Similar results were obtained with ORP150 transgenic mice; when diabetic Akita mice were crossed with ORP150 transgenic mice, insulin sensitivity in Akita mice was significantly increased, which was demonstrated by insulin tolerance test and euglycemic hyperinsulinemic clamp test (Ozawa et al., 2005). IRS-1 tyrosine phosphorylation was markedly increased in Ad-ORP-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. Concomitantly, an increase in Akt serine 473 phosphorylation was observed in Ad-ORP-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. These results indicate that reduction of ER stress enhances insulin signaling which leads to amelioration of glucose tolerance (Fig. 3) (Nakatani et al., 2005).

3.4. The JNK pathway and insulin resistance

It was reported that the JNK pathway is abnormally activated in the liver, muscle, and adipose tissue in obese type 2 diabetic mice and that insulin resistance in obese type 2 diabetic mice is substantially reduced in mice homozygous for a targeted mutation in the JNK1 gene (JNK-KO mice) (Hirosumi, Tuncman, Chang, Karin, & Hotamisligil, 2002). When the JNK-KO mice were placed on a high-fat/high-caloric diet, obese wild type mice developed mild hyperglycemia compared to lean wild type mice. In contrast, blood glucose levels in obese JNK-KO mice was significantly lower compared to those in obese wild type mice. Intraperitoneal insulin tolerance tests showed that hypoglycemic response to insulin in obese wild type mice was lower compared to that in obese JNK-KO mice. Also, intraperitoneal glucose tolerance test revealed a higher degree of hyperglycemia in obese wild type mice than in obese JNK-KO mice. These results indicate that the JNK-KO mice are protected from the development of dietary obesity-induced insulin resistance. Furthermore, targeted mutations in JNK were introduced in genetically obese mice (ob/ob) (Hirosumi et al., 2002). Blood glucose levels in ob/ob-JNK-KO mice were lower compared to those in ob/ob wild type mice, and the ob/ob wild type mice displayed a severe and progressive hyperinsulinemia. Thus, JNK deficiency can provide partial resistance against obesity, hyperglycemia and hyperinsulinemia in both genetic and dietary models of diabetes. Taken together, obese type 2 diabetes is associated with activation of the JNK pathway, and the absence of JNK results in substantial protection from obesity-induced insulin resistance. These results strongly suggest that JNK plays a crucial role in progression of insulin resistance found in type 2 diabetes. It is noted here that there are three isozymes of JNK: JNK1, JNK2, and JNK3, and that only JNK1 has been shown to be implicated in type 2 diabetes (Hirosumi et al., 2002). Thus, it is likely that JNK1 is a crucial mediator of the progression of both insulin resistance and β-cell dysfunction found in type 2 diabetes (Fig. 4). The JNK pathway is known to be activated by several factors such as oxidative stress, free fatty acids (FFAs), tumor necrosis factor-α (TNF-α), all of which are known to be increased under diabetic conditions. Under diabetic conditions, reactive oxygen species (ROS) are produced in various tissues and are involved in the development of insulin resistance as well as the progression of β-cell deterioration. FFAs and TNF-α are also likely to be involved in the development of insulin resistance; levels of FFAs and TNF-α are increased under obese diabetic conditions with insulin resistance, which leads to a further increase in insulin resistance. Thus, we assume that improvement of insulin resistance by the suppression of the JNK pathway was at least in part counterbalancing the deleterious effects of several factors such as oxidative stress, FFAs and TNF-α (Fig. 3).

3.5. The JNK pathway as a potential therapeutic target for insulin resistance

It was also reported that overexpression of dominant-negative (DN) type JNK1 (Ad-DN-JNK) in the liver of obese diabetic C57BL/KsJ-db/db mice dramatically improved insulin resistance and markedly decreased blood glucose levels (Hirosumi et al., 2002). In

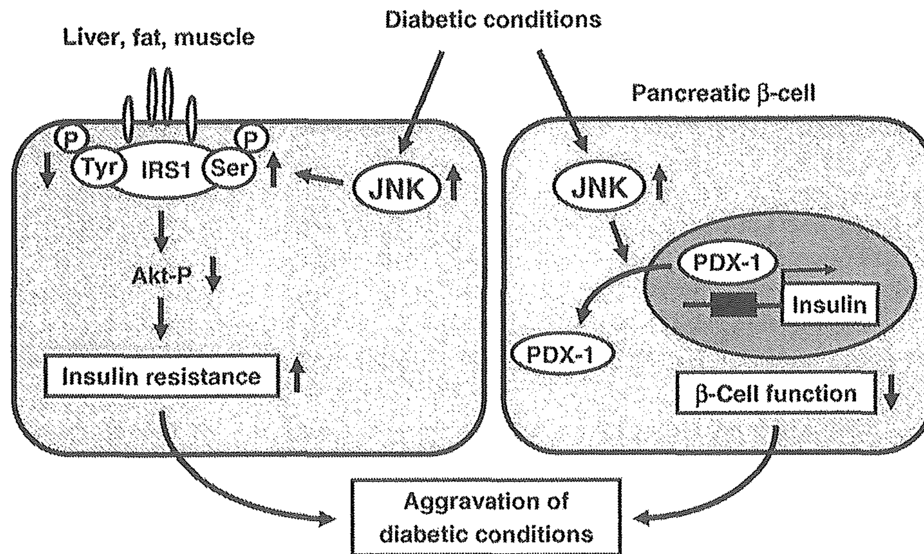

Fig. 4. Role of the JNK pathway in pancreatic β-cell dysfunction and insulin resistance. Activation of the JNK pathway is involved in progression of insulin resistance and deterioration of pancreatic β-cell function.

intraperitoneal insulin tolerance test, the hypoglycemic response to insulin was larger in Ad-DN-JNK-treated db/db mice. Furthermore, in the euglycemic hyperinsulinemic clamp test, glucose infusion rate (GIR) in Ad-DN-JNK-treated mice was higher than that in Ad-GFP-treated mice, indicating that suppression of the JNK pathway in the liver reduces insulin resistance and thus ameliorates glucose tolerance in the db/db mice. Furthermore, hepatic glucose production (HGP) was significantly lower in Ad-DN-JNK-treated mice. In contrast, there was no difference in the glucose disappearance rate (Rd) between these two groups (Nakatani et al., 2004). These results indicate that reduction of insulin resistance and amelioration of glucose tolerance by DN-JNK overexpression are mainly due to suppression of hepatic glucose production. It has been reported that serine phosphorylation of insulin receptor substrate-1 (IRS-1) inhibits insulin-stimulated tyrosine phosphorylation of IRS-1, leading to an increase in insulin resistance (Aguirre et al., 2000). IRS-1 serine 307 phosphorylation was markedly decreased in Ad-DN-JNK-treated mice. An increase in IRS-1 tyrosine and Akt serine 473 phosphorylation was also observed in Ad-DN-JNK-treated mice (Nakatani et al., 2004). Therefore, an increase in IRS-1 serine phosphorylation may be closely associated with the development of insulin resistance induced by JNK overexpression. These results indicate that suppression of the JNK pathway enhances insulin signaling which leads to amelioration of glucose tolerance. Similar effects were observed in high-fat/high-sucrose diet-induced diabetic mice. Conversely, expression of wild type JNK in the liver of normal mice decreased insulin sensitivity (Nakatani et al., 2004). Taken together, these findings suggest that suppression of the JNK pathway in the liver exerts greatly beneficial effects on insulin resistance status and glucose tolerance in both genetic and dietary models of diabetes (Fig. 3).

Protein transduction domains (PTDs) such as the small PTD from the TAT protein of human immunodeficiency virus (HIV-1), the VP22 protein of Herpes simplex virus, and the third α-helix of the homeodomain of Antennapedia, a Drosophila transcription factor, are known to allow various proteins and peptides to be efficiently delivered into cells through the plasma membrane, and thus there has been increasing interest in their potential usefulness for the delivery of bioactive proteins and peptides into cells (Elliott & O'Hare, 1997; Frankel & Pabo, 1988; Noguchi, Kaneto, Weir, & Bonner-Weir, 2003; Schwarze, Ho, Vocero-Akbani, & Dowdy, 1999). It was recently reported that the cell-permeable JNK inhibitory peptide (amino acid sequence: GRK KRR QRR RPP RPK RPT TLN LFP QVP RSQ DT) is effective for the treatment

of diabetes. This peptide is derived from the JNK binding domain of JNK-interacting protein-1 (JIP-1), also known as islet-brain-1 (IB-1), and has been reported to function as a dominant inhibitor of the JNK pathway (Bonny, Oberson, Negri, Sause, & Schorderet, 2001). To convert the minimal JNK-binding domain into a bioactive cell-permeable compound, a 20-amino acid sequence derived from the JNK-binding domain of JIP-1 (RPK RPT TLN LFP QVP RSQ DT) was covalently linked to a 10-amino acid carrier peptide derived from the HIV-TAT sequence (GRK KRR QRR R); then to monitor peptide delivery, this JIP-1-HIV-TAT peptide was further conjugated with fluorescein isothiocyanate (FITC). When this peptide was injected intraperitoneally to C57BL/KsJ-db/db obese diabetic mice, the FITC-conjugated peptide showed fluorescence signals in insulin target organs (liver, fat, muscle) and in insulin secreting tissue (pancreatic islets) (Kaneto et al., 2004). Glucose tolerance in JIP-1-HIV-TAT-FITC-treated mice was significantly ameliorated compared to untreated or the scramble peptide-treated mice. In insulin tolerance test, reduction of blood glucose levels in response to injected insulin was much larger in JIP-HIV-TAT-FITC-treated mice (Kaneto et al., 2004). Furthermore, in the euglycemic hyperinsulinemic clamp test, the steady-state glucose infusion rate (GIR) in JIP-1-HIV-TAT-FITC-treated mice was significantly higher than that in untreated mice, indicating that JIP-1-HIV-TAT-FITC reduces insulin resistance in the db/db mice. Endogenous hepatic glucose production (HGP) and glucose disappearance rate (Rd) in the JNK inhibitory peptide-treated mice was also evaluated. It is noted that Rd reflects glucose utilization in the peripheral tissues. HGP in JIP-1-HIV-TAT-FITC-treated mice was significantly lower than that in untreated mice. In addition, Rd in JIP-1-HIV-TAT-FITC-treated mice was significantly higher than that in untreated mice (Kaneto et al., 2004). These results indicate that JIP-1-HIV-TAT-FITC treatment reduces insulin resistance through decreasing HGP and increasing Rd. IRS-1 serine 307 phosphorylation was decreased in JIP-1-HIV-TAT-FITC-treated mice. An increase of IRS-1 tyrosine phosphorylation was observed in the peptide-treated mice. Concomitantly, increase of Akt serine 473 and threonine 308 phosphorylation was observed in JIP-1-HIV-TAT-FITC-treated mice (Kaneto et al., 2004). In addition, to examine the effect of JIP-1-HIV-TAT-FITC treatment on insulin biosynthesis, we measured insulin mRNA level and content in pancreata of C57BL/KsJ-db/db mice which had been treated every day for 2 weeks with the peptide. Insulin mRNA level and insulin content were significantly higher in the peptide-treated mice. However 3 days after starting treatment with the JNK inhibitory peptide, there was no difference in insulin mRNA levels in pancreas between JIP-1-HIV-TAT-FITC-treated and untreated mice, although blood glucose levels were already decreased 3 days after the treatment and insulin tolerance test showed that insulin resistance was also already reduced. Thus, we assume that the initial target of the peptide treatment is not pancreatic islets, although we cannot totally deny the possibility that the JNK inhibitory peptide exerted some direct effects on the pancreatic islets, because JIP-1-HIV-TAT-FITC is efficiently delivered into pancreatic islets. In conclusion, suppression of the JNK pathway improves insulin resistance and ameliorates glucose intolerance, indicating that the JNK pathway plays a crucial role and could be a potential therapeutic target for diabetes (Fig. 4).

4. Conclusion

The JNK activation is involved in the progression of insulin resistance as well as deterioration of pancreatic β-cell function. Indeed, suppression of the JNK pathway in obese diabetic mice markedly improves insulin resistance and β-cell function and ameliorates glucose tolerance. Taken together, the JNK pathway plays a crucial role in the progression of insulin resistance as well as β-cell dysfunction and thus could be a potential therapeutic target for diabetes (Fig. 4).

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