Nagahara, H., Vocero-Akbani, A. M., Snyder, E. L., Ho, A., Latham, D. G., Lissy, N. A., Becker-Hapak, M., Ezhevsky, S. A., & Dowdy, S. F. (1998). Transduction of full-length TAT fusion proteins into mammalian cells:TAT-p-27^{Kip1} induces cell migration. *Nat Med, 4*, 1449–1452.

Nakatani, Y., Kaneto, H., Kawamori, D., Hatazaki, M., Miyatsuka, T., Matsuoka, T., Kajimoto, Y., Matsuhisa, M., Yamasaki, Y., & Hori, M. (2004). Modulation of the JNK pathway in liver affects insulin resistance status. *J Biol Chem, 279*, 45803–45809.

Nakatani, Y., Kaneto, H., Kawamori, D., Yoshiuchi, K., Hatazaki, M., Matsuoka, T., Ozawa, K., Ogawa, T., Hori, M., Yamasaki, Y., & Matsuhisa, M. (2005). Involvement of ER stress in insulin resistance and diabetes. *J Biol Chem, 280*, 847–851.

Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kaneda, Y., Yorek, M. A., Beebe, D., Oates, P. J., Hammes, H. P., Giardino, I., & Brownlee, M. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature, 404*, 787–790.

Noguchi, H., Kaneto, H., Weir, GC., & Bonner-Weir, S. (2003). PDX-1 protein containing its own Antennapedia-like protein transduction domain can transduce pancreatic duct and islet cells. *Diabetes, 52*, 1732–1737.

Noguchi, H., Matsushita, M., Okitsu, T., Moriwaki, A., Tomizawa, K., Kang, S., Li, S. T., Kobayashi, N., Matsumoto, S., Tanaka, K., Tanaka, N., & Matsui, H. (2004). A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. *Nat Med, 10*, 305–309.

Ohlsson, H., Karlsson, K., & Edlund, T. (1993). IPF1, a homeodomain-containing-transactivator of the insulin gene. *EMBO J, 12*, 4251–4259.

Oyadomari, S., Takeda, K., Takiguchi, M., Gotoh, T., Matsumoto, M., Wada, I., Akira, S., Araki, E., & Mori, M. (2001). Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. *Proc Natl Acad Sci USA, 98*, 10845–10850.

Oyadomari, S., Koizumi, A., Takeda, K., Gotoh, T., Akira, S., Araki, E., & Mori, M. (2002). Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest, 109*, 525–532.

Ozawa, K., Miyazaki, M., Matsuhisa, M., Takano, K., Nakatani, Y., Hatazaki, M., Tamatani, T., Yamagata, K., Miyagawa, J., Kitao, Y., Hori, O., Yamasaki, Y., & Ogawa, S. (2005). The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes. *Diabetes, 54*, 657–663.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, LH., & Hotamisligil, G. S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science, 306*, 457–461.

Peers, B., Leonard, J., Sharma, S., Teitelman, G., & Montminy, M. R. (1994). Insulin expression in pancreatic islet cells relies on cooperative interactions between the helix loop helix factor E47 and the homeobox factor STF-1. *Mol Endocrinol, 8*, 1798–1806.

Petersen, H. V., Serup, P., Leonard, J., Michelsen, BK., & Madsen, O. D. (1994). Transcriptional regulation of the human insulin gene is dependent on the homeodomain protein STF1/IPF1 acting through the CT boxes. *Proc Natl Acad Sci USA, 91*, 10465–10469.

Pick, A., Clark, J., Kubstrup, C., Levisetti, M., Pugh, W., Bonner-Weir, S., & Polonsky, K. (1998). Role of apoptosis in failure of β-cell mass compensation for insulin resistance and β-cell defects in the male Zucker diabetes fatty rats. *Diabetes, 47*, 358–364.

Poitout, V., & Robertson, R. P. (2002). Minireview: Secondary beta-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity. *Endocrinology, 143*, 339–342.

Robertson, R. P., Harmon, J., Tran, P. O., Tanaka, Y., & Takahashi, H. (2003). Glucose toxicity in β-cells: Type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes, 52*, 581–587.

Robertson, R. P. (2004). Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem, 279*, 42351–42354.

Ron, D. (2002). Translational control in the endoplasmic reticulum stress response. *J Clin Invest, 110*, 1383–1388.

Rothbard, J. B., Garlington, S., Lin, Q., Kirschberg, T., Kreider, E., McGrane, P. L., Wender, P. A., & Khavari, P. A. (2000). Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. *Nat Med, 6*, 1253–1257.

Rudich, A., Tirosh, A., Potashnik, R., Hemi, R., Kanety, H., & Bashan, N. (1998). Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes, 47*, 1562–1569.

Sakai, K., Matsumoto, K., Nishikawa, T., Suefuji, M., Nakamura, K., Hirashima, Y., Kawashima, J., Shirotani, T., Ichinose, K., Brownlee, M., & Araki, E. (2003). Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic β-cells. *Biochem Biophys Res Commun, 300*, 216–222.

Schwarze, S. R., Ho, A., Vocero-Akbani, A. M., & Dowdy, S. F. (1999). In vivo protein transduction: Delivery of a biologically active protein into the mouse. *Science, 285*, 1569–1572.

Shi, Y., Vattem, K. M., Sood, R., An, J., Liang, J., Stramm, L., & Wek, R. C. (1998). Identification and characterization of pancreatic eukaryotic initiation factor 2α-subunit kinase, PEK involved in translational control. *Mol Cell Biol, 18*, 7499–7509.

Shi, Y., Taylor, S. I., Tan, S. L., & Sonenberg, N. (2003). When translation meets metabolism: Multiple links to diabetes. *Endocr Rev, 24*, 91–101.

Shimabukuro, M., Zhou, Y. T., Levi, M., & Unger, R. (1998). Fatty acid-induced β cell apoptosis: A link between obesity and diabetes. *Proc Natl Acad Sci USA, 95*, 2498–2502.

Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L., & Habener, J. F. (1997). Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet, 15*, 106–110.

Tanaka, Y., Gleason, C. E., Tran, P. O. T., Harmon, J. S., & Robertson, R. P. (1999). Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci USA, 96*, 10857–10862.

Tanaka, Y., Tran, P. O. T., Harmon, J., & Robertson, R. P. (2002). A role of glutathione peroxidase in protecting pancreatic β cells against oxidative stress in a model of glucose toxicity. *Proc Natl Acad Sci USA, 99*, 12363–12368.

Taniguchi, H., Yamato, E., Tashiro, F., Ikegami, H., Ogihara, T., & Miyazaki, J. (2003). β-Cell neogenesis induced by adenovirus-

mediated gene delivery of transcription factor pdx-1 into mouse pancreas. *Gene Ther, 10*, 15–23.

Tiedge, M., Lortz, S., Drinkgern, J., & Lenzen, S. (1997). Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes, 46*, 1733–1742.

Tirasophon, W., Welihinda, A. A., & Kaufman, R. J. (1998). A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev, 12*, 1812–1824.

Tirosh, A., Potashnik, R., Bashan, N., & Rudich, A. (1999). Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes: A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. *J Biol Chem, 274*, 10595–10602.

Unger, R. H. (1995). Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: Genetic and clinical implications. *Diabetes, 44*, 863–870.

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., & Ron, D. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science, 287*, 664–666.

Waeber, G., Thompson, N., Nicod, P., & Bonny, C. (1996). Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Mol Endocrinol, 10*, 1327–1334.

Wang, X. Z., Harding, H. P., Zhang, Y., Jolicoeur, E. M., Kuroda, M., & Ron, D. (1998). Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J, 17*, 5708–5717.

Wang, H., Maechler, P., Ritz-Laser, B., Hagenfeldt, K. A., Ishihara, H., Philippe, J., & Wollheim, C. B. (2001). Pdx1 level defines pancreatic gene expression pattern and cell lineage differentiation. *J Biol Chem, 276*, 25279–25286.

Watada, H., Kajimoto, Y., Umayahara, Y., Matsuoka, T., Kaneto, H., Fujitani, Y., Kamada, T., Kawamori, R., & Yamasaki, Y. (1996). The human glucokinase gene β-cell-type promoter: An essential role of insulin promoter factor 1 (IPF1)/PDX-1 in its activation in HIT-T15 cells. *Diabetes, 45*, 1478–1488.

Weir, G. C., Laybutt, D. R., Kaneto, H., Bonner-Weir, S., & Sharma, A. (2001). β-Cell adaptation and decompensation during the progression of diabetes. *Diabetes, 50*, 154–159.

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A Crucial Role of MafA as a Novel Therapeutic Target for Diabetes* ♦

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MafA, a recently isolated pancreatic β-cell-specific transcription factor, is a potent activator of insulin gene transcription. In this study, we show that MafA overexpression, together with PDX-1 (pancreatic and duodenal homeobox factor-1) and NeuroD, markedly increases insulin gene expression in the liver. Consequently, substantial amounts of insulin protein were induced by such combination. Furthermore, in streptozotocin-induced diabetic mice, MafA overexpression in the liver, together with PDX-1 and NeuroD, dramatically ameliorated glucose tolerance, while combination of PDX-1 and NeuroD was much less effective. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes.

It is known that the insulin gene is specifically expressed in pancreatic β-cells and that insulin plays a crucial role in maintaining blood glucose levels. It was previously shown that an unidentified β-cell-specific nuclear factor binds to a conserved cis-regulatory element called RIPE3b in the insulin gene promoter region and is likely to function as an important transactivator for the insulin gene (1, 2). Recently, this important transactivator for the insulin gene was identified as MafA, a basic leucine zipper transcription factor (3–6). MafA controls β-cell-specific expression of the insulin gene through a cis-regulatory element called RIPE3b and functions as a potent transactivator for the insulin gene (3–8). During pancreas development, MafA expression is first detected at the beginning of the principal phase of insulin-producing cell production (4), while other important transcription factors such as the pancreatic and duodenal homeobox factor-1 (PDX-1)[1] (8–11) and NeuroD (12, 13) are expressed from the early stage of pancreas development. In addition, while both PDX-1 and NeuroD are expressed in various types of cells in islets, MafA is the only β-cell-specific transactivator for the insulin gene. Thus, the potency of MafA as an insulin gene activator, together with its unique expression in β-cells, raises the likelihood that MafA is a principal factor of β-cell formation and function.

Insulin plays a crucial role in maintaining blood glucose levels, but in the diabetic state, chronic hyperglycemia decreases insulin gene expression and secretion. MafA DNA binding activity is also reduced under diabetic conditions in parallel with the decrease of insulin gene expression (2, 14–16). Although normoglycemia can be efficiently restored by pancreas and islet transplantation, such treatment requires life-long immunosuppressive therapy and is limited by tissue supply (17, 18). Therefore, in exploring new therapeutic methods to replace the reduced insulin in diabetes and to maintain normal glucose tolerance, it is very important to search for ways to enhance insulin gene transcription and to induce insulin-producing cells.

In this study, we show that MafA expression in the liver, together with PDX-1 and NeuroD, markedly induces insulin gene transcription and dramatically ameliorates glucose tolerance in diabetic model animals. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes and imply that such combination should be useful for replacing the reduced β-cell function found in diabetes.

MATERIALS AND METHODS

Gene Transfection and Luciferase Assays—HepG2 cells were grown in Earle's minimal essential medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin sulfate in a humidified atmosphere of 5% CO_2 at 37 °C. The insulin 2 promoter-reporter plasmid (1.0 μg) containing 238-bp 5′-flanking sequences of the rat insulin 2 promoter region (1, 19) and 0.5 μg of the pSV-β-galactosidase control vector (Promega) were co-transfected with 1.0 μg of the MafA, PDX-1, and/or NeuroD expression plasmids (or the empty vectors) using the Lipofectamine reagent (Life Technologies). Forty-eight hours after transfection, cells were harvested for luciferase and β-galactosidase assays. Preparations of cellular extracts were assayed using a luciferase assay system (Promega). For the luciferase assay, light emission was measured with a Monolight 3010 Luminometer (Pharmingen), and β-galactosidase assays were performed with the β-galactosidase enzyme assay system (Promega). The luciferase results were normalized with respect to transfection efficiency assessed from the results of the β-galactosidase assays.

Preparation of Recombinant Adenoviruses Expressing MafA, PDX-1, and NeuroD (Ad-MafA, Ad-PDX-1, and Ad-NeuroD)—Recombinant adenoviruses expressing MafA, PDX-1, and NeuroD were prepared using the AdEasy system (kindly provided by Dr. Bert Vogelstein, Johns Hopkins Oncology Center) (20). In brief, the encoding region of MafA, PDX-1, and NeuroD was cloned into a shuttle vector pAdTrack-CMV. To produce homologous recombination, 1.0 μg of linearized plasmid containing MafA, PDX-1, and NeuroD and 0.1 μg of the adenoviral backbone plasmid, pAdEasy-1, were introduced into electrocompetent *Escherichia coli* BJ5183 cells by electroporation (2,500 V, 200 ohms, 25 microfarads). The resultant plasmids were then re-transformed into *E. coli* XL-Gold Ultracompetent Cells (Stratagene, La Jolla, CA). The plasmids were linearized with PacI and then transfected into the adenovirus packaging cell line 293 using Lipofectamine (Invitrogen). Ten

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[1] The abbreviations used are: PDX-1, pancreatic and duodenal homeobox factor-1; Ad, adenovirus; GFP, green fluorescent protein; pfu, plaque-forming unit; STZ, streptozotocin; MOPS, 4-morpholinepropanesulfonic acid; PBS, phosphate-buffered saline; ABC, avidin-biotin complex.

days after transfection, the cell lysate was collected from the 293 cells. The cell lysate was added to fresh 293 cells and when most of the cells were killed by the adenovirus infection and detached, the cell lysate was obtained again (this process was repeated three times). The control adenovirus expressing green fluorescent protein (Ad-GFP) was prepared in the same manner. The adenovirus titers were further increased up to 1×10^{10} plaque forming units (pfu)/ml using the Adeno-X™ virus purification kit (Clontech). The virus titers were estimated using the Adeno-X™ titer kit (Clontech).

Induction of Hyperglycemia by Streptozotocin (STZ) and Treatment with Recombinant Adenovirus—C57BL6 male mice (8 weeks old) (Japan SLC, Hamamatsu, Japan) were made diabetic by intraperitoneal injection of STZ (220 mg/kg) (Sigma), freshly dissolved in citrate buffer (pH 4.5). One week after STZ injection, mice were injected with 100 μl of Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1×10^{10} pfu/ml) into the cervical vein. It is noted that the adenovirus injected from the cervical vein is known to be trapped only in the liver after its systematic circulation. After adenovirus injection, nonfasting blood glucose levels were measured regularly with a portable glucose meter (Precision QID, Medisense Inc., St. Charles, MA) after tail snipping. For measurement of plasma insulin levels, nonfasting blood samples were collected into heparinized capillary tubes, and plasma insulin levels were determined using an insulin-EIA test kit (Glazyme).

Glucose Tolerance Tests—After overnight fast, mice were injected intraperitoneally with glucose (2.0 g/kg of body weight). Blood samples were taken at various time points (0–120 min), and blood glucose levels were determined as described above.

Northern Blot Analysis—Ten micrograms of total RNA isolated from freeze-clamped liver tissues were electrophoresed on 1.0% formaldehyde-denatured agarose gel in 1 × MOPS running buffer and then transferred overnight to a Hybond-N^+ membrane (Amersham Biosciences). The insulin probe was labeled with [α-^{32}P]dCTP using the Rediprime labeling system kit (Amersham Biosceinces). After overnight hybridization with a ^{32}P-labled probe at 42 °C, the membranes were washed in 2 x saline/sodium phosphate/EDTA, 0.1% SDS at 42 °C. Kodak XAR film was exposed with an intensifying screen at −80 °C.

Reverse Transcriptase-PCR Analysis—Total RNA was extracted from frozen tissues using TRIzol (Invitrogen). After quantification by spectrophotometry, 2.5 μg of RNA was heated at 85 °C for 3 min and then reverse-transcribed into cDNA in a 25-μl solution containing 200 units of Superscript II RNase H^- reverse transcriptase (Invitrogen), 50 ng of random hexamers (Invitrogen), 160 μM dNTP, and 10 mM dithiothreitol. The reaction consisted of 10 min at 25 °C, 60 min at 42 °C, and 10 min at 95 °C. Polymerization reactions were performed with a Perkin-Elmer 9700 Thermocycler using a 50-μl reaction volume containing 3 μl of cDNA (20 ng of RNA equivalents), 5 units of AmpliTaq Gold DNA polymerase (PerkinElmer Life Sciences), 1.5 mM $MgCl_2$, 160 μM cold dNTPs, and 10 pmol of the appropriate oligonucleotide primers. The oligonucleotide primers were as follows: insulin 1 (370 bp), GAC CAG CTA TAA TCA GAG ACC (forward) and AGT TGC AGT AGT TCT CCA GCT G (reverse); insulin 2 (388 bp), AGC CCT AAG TGA TCC GCT ACA A (forward) and AGTTGCAGTAGTTCTCCAGCTG (reverse); glucokinase (islet type) (208 bp), TGG ATG ACA GAG CCA GGA TGG (forward) and ACT TCT GAG CCT TCT GGG GTG (reverse); SUR-1 (267 bp), CCA GAC CAA GGG AAG ATT CA (forward) and GTC CTG TAG GAT GAT GGA CA (reverse); Kir6.2 (218 bp), CCT GAG GAA TAT GTG CTG AC (forward) and CAC AGG AAG GAC ATG GTG AA (reverse); glucagon (205 bp), ACA GAG GAG AAC CCC AGA TC (forward) and CAT CAT GAC GTT TGG CAA TG (reverse); somatostatin (226 bp), AGT TTC TGC AGA AGT CTC TGG (forward) and AAG TTC TTG CAG CCA GCT TTG (reverse); pancreatic polypeptide (194 bp), ACA GGA TGG CCG TCG CAT ACT (forward) and GGC CTG GTC AGT GTG TTG ATG (reverse). The thermal cycle profile employed a 10-min denaturing step at 94 °C followed by 32 cycles (1 min of denaturation at 94 °C, 1 min of annealing at 55 °C, and 1 min of extension at 72 °C) and an extension step of 10 min at 72 °C. The products were then separated by agarose gel electrophoresis.

Measurement of Insulin Content and Plasma Insulin Levels—For measurement of insulin content, the liver was excised, and insulin content in the tissue was determined using an insulin-EIA test kit (Glazyme) with mouse insulin as the standard. The data were normalized with respect to protein concentration in the extract, which was measured using a protein assay (Bio-Rad). For measurement of plasma insulin levels, blood samples were collected into heparinized capillary tubes, and plasma insulin levels were determined using an insulin-EIA test kit (Glazyme).

Immunohistochemical Analyses—The mice were anesthetized using sodium pentobarbital. After a midline abdominal incision, pancreata were removed from the mice and fixed overnight with 4% paraformaldehyde in PBS buffer. Fixed tissues were routinely processed for paraffin embedding and ~4-μm sections were prepared and mounted on slides. Before incubation with antibodies, the mounted sections were rinsed with PBS three times. For detection of MafA, PDX-1, and NeuroD, the avidin-biotin complex (ABC) method was performed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). After treatment with target retrieval solution (Dako, Glostrup, Denmark) at 90 °C for 5 min, the mounted sections were incubated overnight with rabbit anti-MafA antibody, rabbit anti-PDX-1 antiserum (21), and goat anti-NeuroD antibody (N-19) (Santa Cruz Biotechnology) diluted 1:1000 in PBS containing 1% bovine serum albumin. This was followed by 1 h incubation with biotinylated anti-rabbit IgG (for MafA and PDX-1) or anti-goat IgG (for NeuroD) (Vector Laboratories) diluted 1:200. The sections were then incubated with ABC reagent for 1 h and positive reactions were visualized by incubation with the peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride (Zymed

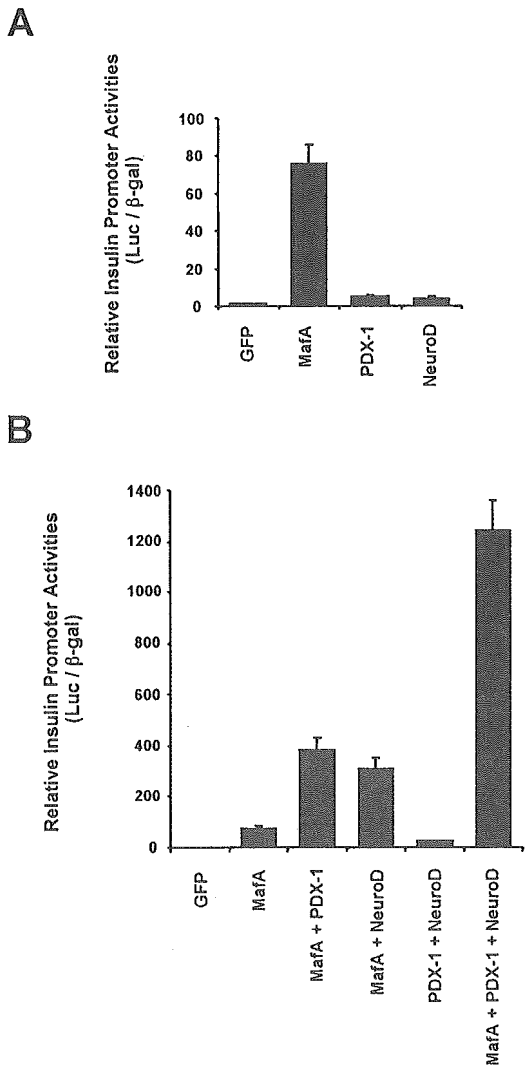

FIG. 1. **MafA expression, together with PDX-1 and NeuroD, markedly induces insulin promoter activity.** *A*, the rat insulin promoter-reporter (luciferase) plasmid and pSV-β-galactosidase control vector were co-transfected into HepG2 cells with the MafA, PDX-1, or NeuroD expression plasmids (or the empty vector); 2 days after the transfection, luciferase and β-galactosidase assays were performed. *B*, the rat insulin promoter-reporter (luciferase) plasmid and pSV-β-galactosidase control vector were was co-transfected with MafA expression plasmid, together with PDX-1 and/or NeuroD expression plasmid; 2 days after the transfection, luciferase and β-galactosidase assays were performed. The luciferase results were normalized with respect to the transfection efficiency assessed from the results of the β-galactosidase assays. Data are expressed as mean ± S.E. with the basal insulin promoter activity being arbitrarily set at 1 (n = 4).

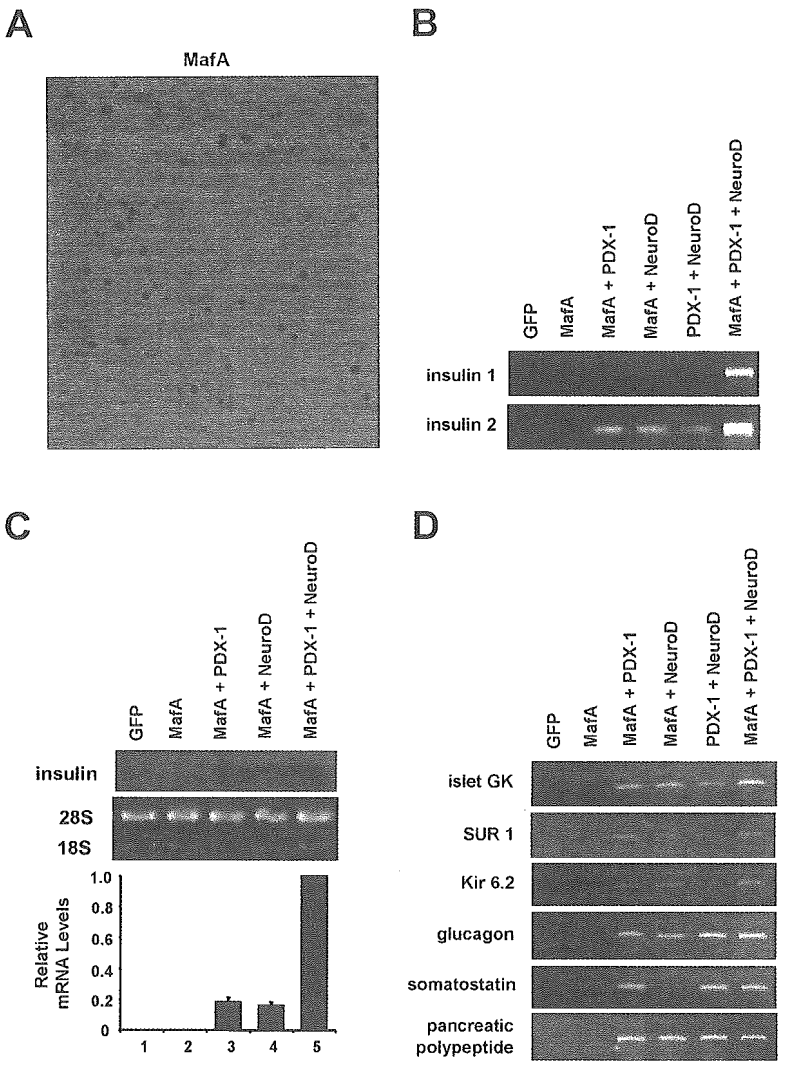

FIG. 2. **MafA expression in the liver, together with PDX-1 and NeuroD, markedly induces insulin gene expression.** *A*, male C57BL6 mice were injected with 100 μl of Ad-MafA (1×10^{10} pfu/ml) into the cervical vein. This panel shows a representative liver after exposure to Ad-MafA. MafA protein expression is clearly detected in the liver 3 days after infection with Ad-MafA. *B*, male C57BL6 mice were injected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1×10^{10} pfu/ml) into the cervical vein; 3 days after the injection we examined mRNA expression levels of insulin 1 and 2 gene. Similar results were obtained in three independent experiments. *C*, male C57BL6 mice were injected with 100 μl of Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1×10^{10} pfu/ml) into the cervical vein, and 3 days after the injection we examined insulin mRNA expression by Northern blot analysis. Data are expressed as mean ± S.E. with the insulin mRNA levels after treatment with Ad-MafA plus Ad-PDX-1 plus Ad-NeuroD being arbitrarily set at 1.0 (n = 3). *D*, expression of various pancreas-related gene mRNA (islet-type glucokinase (*GK*), sulfonylurea receptor 1 (*SUR 1*), Kir 6.2, glucagon, somatostatin, and pancreatic polypeptide) in male C57BL6 mice infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1×10^{10} pfu/ml). Similar results were obtained in three independent experiments.

Laboratories Inc.). For detection of insulin, the mounted sections were incubated overnight with guinea pig polyclonal anti-insulin antibody (Dako) diluted 1:1,000 in PBS containing 1% bovine serum albumin. This was also followed by 1-h incubation with biotinylated anti-rabbit IgG diluted 1:200. The sections were then incubated with ABC reagent for 1 h, and positive reactions were visualized by incubation with the peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride.

RESULTS

MafA Expression in the Liver, together with PDX-1 and NeuroD, Markedly Induces Insulin Gene Expression—To evaluate the effect of MafA, PDX-1, and NeuroD expression on insulin gene transcription in the liver, we examined insulin gene promoter activity in HepG2 cells after transfection of each expression plasmid. As shown in Fig. 1*A*, basal insulin promoter activity was increased by MafA alone (~80-fold increase), which was much more significant after overexpression of PDX-1 or NeuroD alone (~5-fold increase). As shown in Fig. 1*B*, insulin promoter activity was further increased by MafA in the presence of PDX-1 or NeuroD (~300- and ~400-fold increase, respectively). Furthermore, MafA, together with PDX-1 plus NeuroD, drastically increased insulin promoter activity (~1,200-fold increase). These results clearly show that MafA, PDX-1, and NeuroD exert strong synergistic effect on insulin promoter activity.

Next, to evaluate the effect of ectopic expression of MafA, PDX-1, and NeuroD in the liver, we prepared MafA-, PDX-1-, and NeuroD-expressing adenoviruses (Ad-MafA, Ad-PDX-1, and Ad-NeuroD) and a control adenovirus (Ad-GFP), then injected each adenovirus into the cervical vein of 8-week-old male C57BL6 mice. To confirm that infected adenoviruses can express the target protein in the liver, we performed immunostaining for MafA, PDX-1, and NeuroD. As shown in Fig. 2*A*, MafA was clearly detected in the liver 3 days after infection with Ad-MafA. Similarly, PDX-1 and NeuroD were also clearly detected in the liver after infection with Ad-PDX-1 and Ad-NeuroD, respectively (data not shown). Also, to confirm that the adenovirus infected only the liver, we examined GFP expression in various tissues (brain, heart, lung, liver, spleen, pancreas, kidney, fat, and muscle). GFP was expressed only in the liver, but not in any other tissues (data not shown). After the adenovirus infection, we evaluated insulin mRNA levels by reverse transcriptase-PCR. Since it is known that rodents have two insulin genes: insulin 1 and insulin 2, we examined which insulin gene is induced by such adenoviruses. As shown in Fig. 2*B*, neither insulin 1 nor 2 gene expression was induced by Ad-MafA alone, but both insulin 1 and 2 were induced by Ad-MafA plus Ad-PDX-1 or Ad-MafA plus Ad-NeuroD. It is noted that insulin 1 gene expression was induced only in the presence of MafA, suggesting that MafA plays a crucial role in inducing insulin gene expression. Larger amounts of insulin 1 and 2 mRNA expression were clearly observed in the liver after the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD).

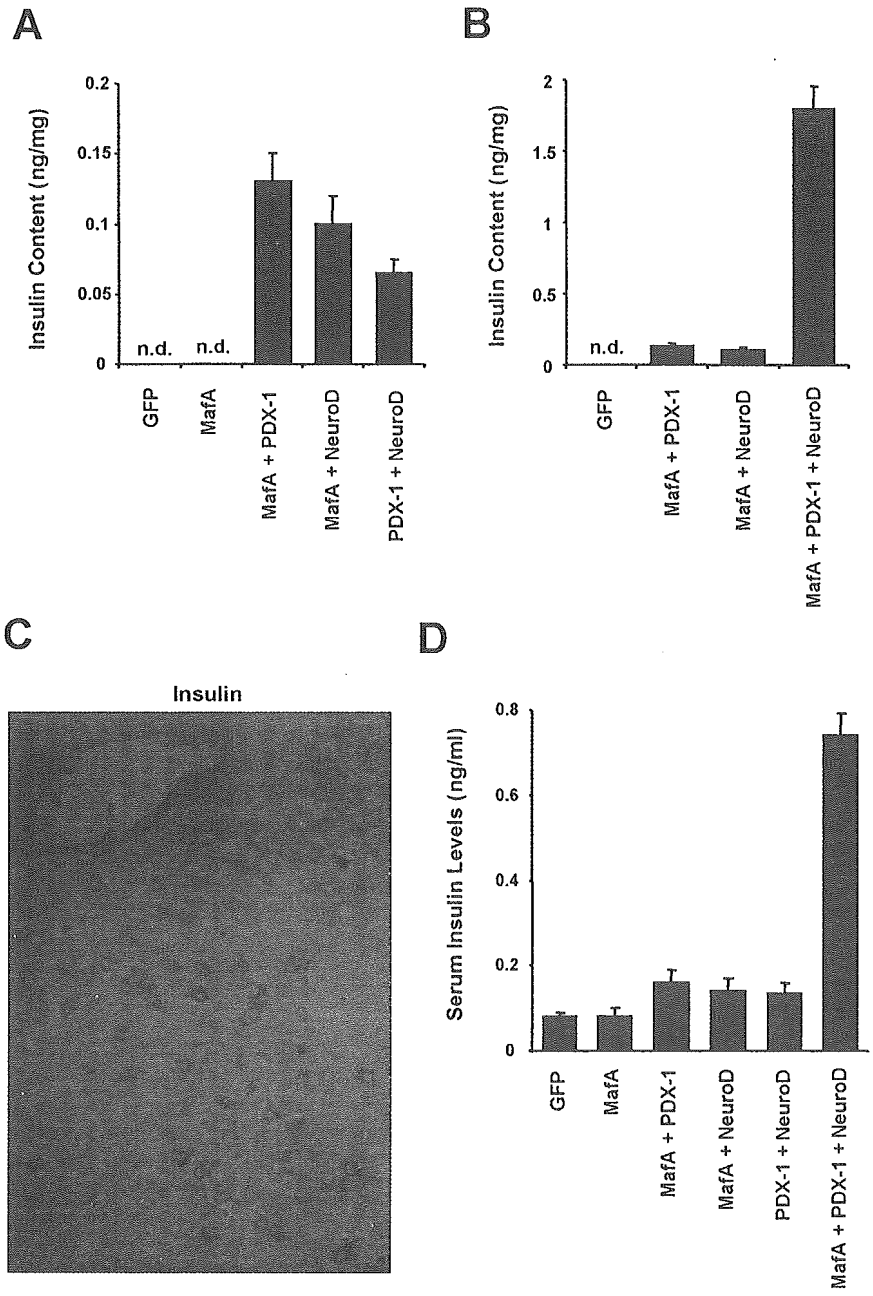

FIG. 3. **MafA expression in the liver, together with PDX-1 and NeuroD, markedly induces insulin-producing cells in the liver.** *A*, insulin content in the liver in C57BL6 mice infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP. Data are expressed as mean ± S.E. (n = 3). *n.d.*, not detected. *B*, insulin content in the liver in C57BL6 mice infected with Ad-MafA, together with Ad-PDX-1 and/or Ad-NeuroD. Data are expressed as mean ± S.E. (n = 3). *n.d.*, not detected. *C*, immunostaining for insulin in the liver in C57BL6 mice infected with Ad-MafA, Ad-PDX-1, and Ad-NeuroD. Several insulin-positive cells (brown cells) can be clearly observed in the liver. Similar results were obtained in three independent experiments. *D*, male C57BL6 mice were made diabetic by intraperitoneal injection of streptozotocin (STZ) (220 mg/kg) and 1 week later were infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP. After a 6-h fast, we determined serum insulin levels in the mice. Data are expressed as mean ± S.E. (n = 3).

The effect of the triple infection was more profound compared with that of Ad-PDX-1 plus Ad-NeuroD infection. We further evaluated insulin mRNA levels by Northern blot analysis. As shown in Fig. 2*C*, insulin mRNA expression was detected at all 3 days after the injection of Ad-MafA plus Ad-PDX-1 or Ad-MafA plus Ad-NeuroD. Furthermore, a much larger amount of insulin mRNA expression was induced by the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD).

In addition, to examine various pancreas-related gene expression caused by ectopic expression of MafA, PDX-1, and/or NeuroD, we evaluated the mRNA expression of various pancreas-related genes. First, we examined the expression of β-cell-related genes: islet-type glucokinase, sulfonylurea receptor 1, and Kir6.2. As shown in Fig. 2*D*, mRNA expressions of various β-cell-related genes such as glucokinase, SUR1, and Kir6.2 were detected in the liver after treatment with Ad-MafA in the presence of PDX-1 and/or NeuroD but not detected after treatment with Ad-MafA alone. Similarly, mRNA expressions of various endocrine hormones such as glucagon and pancreatic polypeptide were also detected in the liver after treatment with Ad-MafA in the presence of PDX-1 and/or NeuroD but not detected after treatment with Ad-MafA alone. Somatostatin mRNA expression was detected only in the presence of PDX-1, highlighting the crucial role of PDX-1 in somatostatin gene expression.

MafA Expression in the Liver, together with PDX-1 and NeuroD, Markedly Induces Insulin-producing Cells in the Liver— To examine whether insulin protein was synthesized and stored in the liver, we examined insulin protein expression in the liver after treatment with such adenoviruses. First, we measured insulin content in the liver after the adenovirus infection. As shown in Fig. 3*A*, insulin content was not detected in the liver treated with Ad-MafA alone but was detected after treatment with Ad-MafA in the presence of PDX-1 or NeuroD. Insulin content in the liver induced by MafA in the presence of PDX-1 or NeuroD was even larger than that by PDX-1 plus

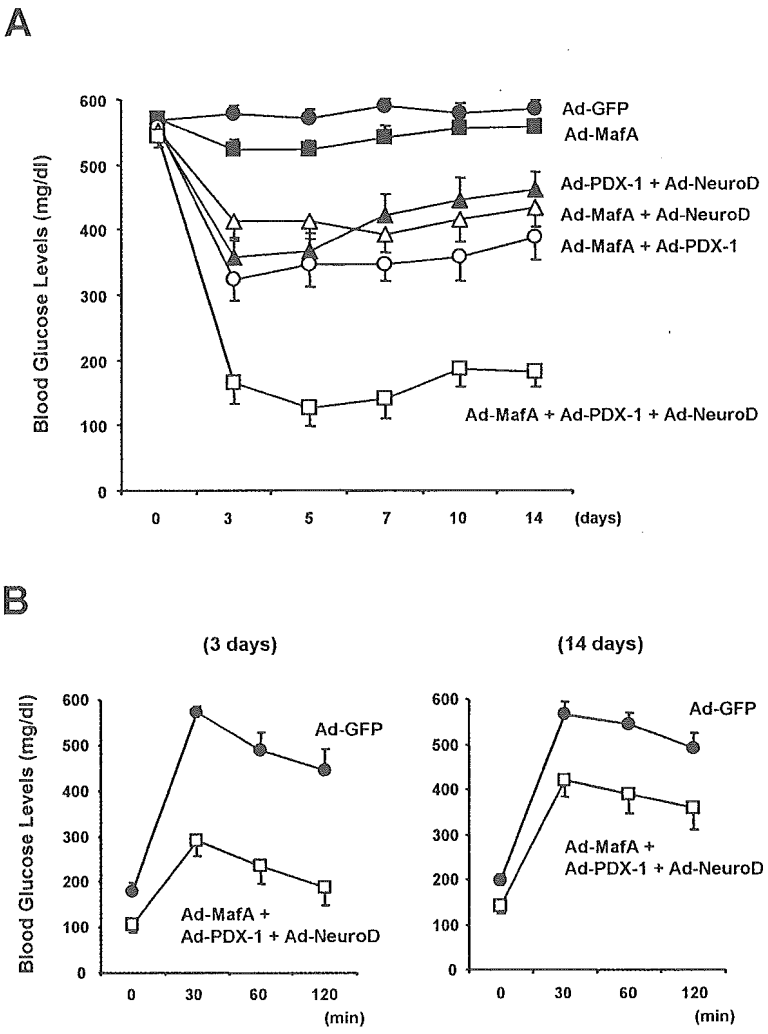

FIG. 4. **MafA expression in the liver, together with PDX-1 and NeuroD, dramatically ameliorates glucose tolerance in diabetic model animals.** *A*, male C57BL6 mice were made diabetic by intraperitoneal injection of STZ (220 mg/kg) and 1 week later were infected with 100 μl of Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1×10^{10} pfu/ml). Nonfasting blood glucose levels were measured with a portable glucose meter after tail snipping 0–14 days after the adenovirus infection. Data are expressed as mean ± S.E. ($n = 5$). *B*, at 3 and 14 days after the adenovirus infection, intraperitoneal glucose tolerance tests were done. After an overnight fast, STZ-induced diabetic C57BL6 mice were injected intraperitoneally with 1.0 g/kg body weight of glucose. Blood samples were taken at various time points (0–120 min) and used to measure blood glucose levels. Data are expressed as mean ± S.E. ($n = 4$).

NeuroD. Also, as shown in Fig. 3*B*, insulin content in the liver was markedly increased after the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). In addition, to reconfirm insulin protein expression in the liver, we performed immunostaining for insulin. As shown in Fig. 3*C*, many insulin-positive cells (brown cells) were clearly observed in the liver after the triple infection. Such effects were not observed after any double infection (Ad-MafA plus Ad-PDX-1, Ad-MafA plus Ad-NeuroD, or Ad-PDX-1 plus Ad-NeuroD) (data not shown).

Furthermore, to determine whether the produced insulin is secreted into the bloodstream, we measured serum insulin levels after the adenovirus infection. We injected 220 mg/kg STZ into C57BL6 mice, and 1 week later we treated the mice with the adenoviruses. As shown in Fig. 3*D*, serum insulin levels were moderately increased after treatment with Ad-MafA plus Ad-PDX-1 or Ad-MafA plus Ad-NeuroD and were markedly increased after the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). Taken together, MafA expression, together with PDX-1 and NeuroD, drastically induces insulin content (Fig. 3, *A–C*) and secretion (Fig. 3*D*), in addition to induction of insulin promoter activity (Fig. 1, *A* and B) and mRNA expression (Fig. 2, *B* and C).

MafA Expression in the Liver, together with PDX-1 and NeuroD, Dramatically Ameliorates Glucose Tolerance in Diabetic Model Animals—We examined whether hepatic insulin production induced by combination of MafA, PDX-1, and NeuroD is capable of controlling blood glucose levels in STZ-induced diabetic mice. As shown in Fig. 4*A*, 3 days after adenovirus injection, blood glucose levels were decreased by MafA plus PDX-1, MafA plus NeuroD, or PDX-1 plus NeuroD, although no such effect was observed after infection of Ad-MafA alone. Furthermore, blood glucose levels were dramatically decreased by the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). The effects of triple infection were much more pronounced compared with those in any single or double infection. It is noted that, despite the marked effects of MafA expression, together with PDX-1 and NeuroD, the mice did not become hypoglycemia. In addition, we performed the intraperitoneal glucose tolerance test at 3 and 14 days after the adenovirus injection. As shown in Fig. 2*B*, there was a marked difference in glucose tolerance at any time point after glucose load between Ad-GFP-treated mice and the mice treated with Ad-MafA, Ad-PDX-1, and Ad-NeuroD.

DISCUSSION

In this study we examined the effects of adenovirus-mediated expression of a recently identified pancreatic β-cell-specific transcription factor MafA on insulin gene expression and glucose tolerance with and without PDX-1 and/or NeuroD and found that MafA overexpression, together with PDX-1 and NeuroD, drastically induces insulin production in the liver (Figs. 2 and 3) and ameliorates glucose tolerance in diabetic animal models (Fig. 4). The marked effects of MafA expression, together with PDX-1 and NeuroD, on insulin production and glucose tolerance indicate that MafA plays an important role in inducing insulin-producing cells and thus should be a novel therapeutic target for diabetes

and that combination therapy should be very efficient and useful for replacing the reduced insulin biosynthesis found in diabetes and for amelioration of glucose tolerance. In addition, despite the marked effects of MafA expression, together with PDX-1 and NeuroD, the mice did not become hypoglycemia. These results imply that some glucose-mediated regulation of insulin production and/or secretion is achieved by the triple infection.

There are several possible reasons to explain why the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD) is much more effective compared with any single or double infection. One possibility is that marked induction of insulin mRNA expression by the triple infection is simply due to increase of insulin promoter activity because insulin promoter activity *per se* is most significantly increased by the triple transfection (Fig. 1*A*). Another is that the transcription partners are recruited to the insulin promoter region by MafA, together with PDX-1 and NeuroD, which would enable these transcription factors to exert strong synergistic effects and to markedly induce insulin gene expression. Both PDX-1 and NeuroD are known to associate functionally with the co-activator p300 and thereby exert strong effects on the insulin gene transcription (22–26). Thus, although not examined in this study, we assume that some transcriptional partner such as p300 contributes to the marked effects of triple overexpression on insulin gene expression. In addition, since p300 is known to interact with transcriptional adaptors such as a particular histone acetyltransferase and to be involved in chromatin remodeling complexes (27, 28), it is possible that recruited p300 to the insulin gene promoter region facilitates histone acetylation and thus contributes to the marked effects of triple overexpression on insulin gene expression.

In conclusion, MafA overexpression, together with PDX-1 and NeuroD, drastically induces insulin production in the liver and ameliorates glucose intolerance in diabetic animal models, indicating that MafA plays an important role in inducing insulin-producing cells and thus should be a novel therapeutic target for diabetes and that combination therapy should be very efficient and useful for replacing the reduced insulin biosynthesis found in diabetes and for amelioration of glucose intolerance.

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REFERENCES

1. Sharma, A., and Stein, R. (1994) *Mol. Cell. Biol.* **14,** 871–879
2. Sharma, A., Fusco-DeMane, D., Henderson, E., Efrat, S., and Stein, R. (1995) *Mol. Endocrinol.* **9,** 1468–1488
3. Matsuoka, T. A., Zhao, L., Artner, I., Jarrett, H. W., Friedman, D., Means, A., and Stein, R. (2003) *Mol. Cell. Biol.* **23,** 6049–6062
4. Matsuoka, T. A., Artner, I., Henderson, E., Means, A., Sander, M., and Stein, R. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101,** 2930–2933
5. Olbrot, M., Rud, J., Moss, L. G., and Sharma, A. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 6737–6742
6. Kataoka, K., Han, S. I., Shioda, S., Hirai, M., Nishizawa, M., and Handa, H. (2002) *J. Biol. Chem.* **277,** 49903–49910
7. Kajihara, M., Sone, H., Amemiya, M., Katoh, Y., Isogai, M., Shimano, H., Yamada, N., Takahashi, S. (2003) *Biochem. Biophys. Res. Commun.* **312,** 831–842
8. Kataoka, K., Shioda, S., Ando, K., Sakagami, K., Handa, H., and Yasuda, K. (2004) *J. Mol. Endocrinol.* **32,** 9–20
9. Miller, C. P., McGehee, R. E., and Habener, J. F. (1994) *EMBO J.* **13,** 1145–1156
10. Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S., and Montminy, M. R. (1993) *Mol. Endocrinol.* **7,** 1275–1283
11. Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) *Nature* **37,** 606–609
12. Naya, F. J., Stellrecht, C. M. M., and Tsai, M.-J. (1995) *Genes Dev.* **9,** 1009–1019
13. Naya, F. J., Huang, H., Qiu, Y., Mutoh, H., DeMayo, F., Leiter, A. B., and Tsai, M.-J. (1997) *Genes Dev.* **11,** 2323–2334
14. Poitout, V., Olson, L. K., and Robertson, R. P. (1996) *J. Clin. Invest.* **97,** 1041–1046
15. Moran, A., Zhang, H. J., Olson, L. K., Harmon, J. S., Poitout, V., and Robertson, R. P. (1997) *J. Clin. Invest.* **99,** 534–539
16. Robertson, R. P. (2004) *J. Biol. Chem.* **279,** 42351–42354
17. Robertson, R. P. (2004) *N. Engl. J. Med.* **350,** 694–705
18. Weir, G. C., and Bonner-Weir, S. (1997) *Diabetes* **46,** 1247–1256
19. Robinson, G. L. W. G., Peshavaria, M., Henderson, E., Shieh, S.-Y., Tsai, M.-J., Teitelman, G., and Stein, R. (1994) *J. Biol. Chem.* **269,** 2452–2460
20. He, T.-C., Zhou, S., DaCosta, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 2509–2514
21. Watada, H., Kajimoto, Y., Umayahara, Y., Matsuoka, T., Kaneto, H., Fujitani, Y., Kamada, T., Kawamori, R., and Yamasaki, Y. (1996) *Diabetes* **45,** 1478–1488
22. Qiu, Y., Sharma, A., and Stein, R. (1998) *Mol. Cell. Biol.* **18,** 2957–2964
23. Mutoh, H., Naya, F. J., Tsai, M. J., and Leiter, A. B. (1998) *Genes Dev.* **12,** 820–830
24. Sharma, A., Moore, M., Marcora, E., Lee, J. E., Qiu, Y., Samaras, S., and Stein, R. (1999) *Mol. Cell. Biol.* **19,** 704–713
25. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2002) *Mol. Cell. Biol.* **22,** 412–420
26. Stanojevic, V., Habener, J. F., and Thomas, M. K. (2004) *Endocrinology* **145,** 2918–2928
27. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2004) *J. Biol. Chem.* **279,** 9796–9802
28. Mosley, A. L., Corbett, J. A., and Ozcan, S. (2004) *Mol. Endocrinol.* **18,** 2279–2290

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REVIEW

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Oxidative stress, ER stress, and the JNK pathway in type 2 diabetes

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Abstract Pancreatic β-cell dysfunction and insulin resistance are observed in type 2 diabetes. Under diabetic conditions, oxidative stress and ER stress are induced in various tissues, leading to activation of the JNK pathway. This JNK activation suppresses insulin biosynthesis and interferes with insulin action. Indeed, suppression of the JNK pathway in diabetic mice improves insulin resistance and ameliorates glucose tolerance. Thus, the JNK pathway plays a central role in pathogenesis of type 2 diabetes and may be a potential target for diabetes therapy.

Keywords Oxidative stress · ER stress · JNK pathway · Insulin biosynthesis · Insulin resistance

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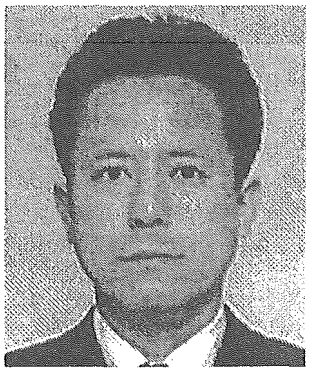

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Abbreviations DN: Dominant-negative · ER: Endoplasmic reticulum · IRS-1: Insulin receptor substrate 1 · JIP-1: JNK-interacting protein-1 · JNK: c-Jun N-terminal kinase · PERK: Pancreatic ER kinase (or PKR-like kinase) · PDX-1: Pancreatic and duodenal homeobox factor-1 · ROS: Reactive oxygen species · TNF-α: Tumor necrosis factor-α · XBP-1: X-box-binding protein-1

Introduction

The development of type 2 diabetes is associated with a combination of pancreatic β-cell dysfunction and insulin resistance [1, 2]. 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 [3–7]. This process is called "glucose toxicity". 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 diabetes.

Oxidative stress and pancreatic β-cell dysfunction

Oxidative stress is provoked in various tissues under diabetic conditions, and is involved in the development of diabetic complications [8–13]. Pancreatic β-cells are also thought to be a target of oxidative stress-mediated tissue damage [14–26]. β-cells express the high K_m glucose transporter GLUT2 and thereby display highly efficient glucose uptake when exposed to high glucose concentrations. Indeed, it has been shown that expression of the oxidative stress markers 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxy-2,3-nonenal (4-HNE) is increased in islets under diabetic conditions [12, 13]. In addition, due to

their relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase [27, 28], β-cells are rather vulnerable to oxidative stress. 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 [14, 15], the mitochondrial electron transport chain [10, 11], and the hexosamine pathway [21]. In diabetic animals, glycation is observed in various tissues and organs, and various kinds of glycated proteins are produced through the glycation reaction. During this reaction which in turn produces Schiff bases, Amadori products and advanced glycosylation end products (AGE), ROS are also produced [14]. Superoxide anions(O_2^-) are initially produced, and then hydrogen peroxide (H_2O_2) and hydroxyl radicals are produced. The electron transport chain in the mitochondria is also likely to be an important pathway for the production of ROS (Fig. 1). In the electron transport chain, the superoxide anion (O_2^-) is initially produced. It has been suggested that mitochondrial overwork, which results in an induction of ROS generation, is a potential mechanism causing the impairment of the first phase of glucose-stimulated insulin secretion found in the early stage of diabetes [23] as well as diabetic complications [10, 11].

As shown previously, the glycation reaction suppresses transcription of the insulin gene in β-cells by provoking oxidative stress. When β-cell-derived HIT cells were exposed to D-ribose, a strong reducing sugar, insulin gene promoter activity and mRNA expression were suppressed, whereas no such changes were observed for the β-actin gene [15]. These effects were neutralized by aminoguanidine, an inhibitor of the glycation reaction, or *N*-acetyl-L-cysteine (NAC), an antioxidant, indicating that D-ribose suppresses insulin gene transcription by provoking oxidative stress via the glycation reaction. Pancreatic and duodenal homeobox factor-1 (PDX-1), also known as IDX-1/STF-1/IPF1 [29–31], is a member of the homeodomain-containing transcription factor family. PDX-1 is expressed in the pancreas and the duodenum and plays a crucial role in pancreatic development [32–40], β-cell differentiation/regeneration [41–51], and in maintaining normal β-cell function by regulating multiple important β-cell genes, including insulin, GLUT2, and glucokinase [52–60]. Clinically, mutations in PDX-1 are known to cause some cases of maturity-onset diabetes of the young (MODY) [61]. The DNA-binding activity of PDX-1 is rather sensitive to oxidative stress, which may explain the reduction in the insulin gene promoter activity by oxidative stress; when HIT cells were exposed to D-ribose, PDX-1 binding to the insulin gene was markedly reduced, which was prevented by aminoguanidine or NAC [15]. Taken together, chronic hyperglycemia suppresses insulin biosynthesis and secretion by provoking oxidative stress through various pathways, accompanied by a reduction of PDX-1 DNA binding activity (Fig. 2).

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 (NAC plus vitamins C and E) and the effects were assessed [16]. The antioxidant treatment resulted in retained glucose-stimulated insulin secretion and moderately decreased blood glucose levels. The β-cell mass was significantly greater in the mice treated with the antioxidants. Antioxidant treatment also suppressed apoptosis in the β-cell population without changing the rate of β-cell proliferation. The insulin content and insulin mRNA levels were also preserved by the antioxidant treatment. Furthermore, PDX-1 expression was more clearly visible in the nuclei of islet cells after the antioxidant treatment [16]. Similar effects were observed with Zucker diabetic fatty (ZDF) rats, another animal model of type 2 diabetes [17]. Taken together, these data indicate that antioxidant treatment can protect β-cells against glucose toxicity. In addition, as a step to clinical trial of antioxidant for type 2 diabetes, we investigated the possible anti-diabetic effects of probucol, an antioxidant widely used as an anti-hyperlipidemic agent, on preservation of β-cell function in diabetic C57BL/KsJ-*db/db* mice [13]. Immunostaining for oxidative stress markers such as 4-hydroxy-2-nonenal (HNE)-modified proteins and heme oxygenase-1 revealed that probucol treatment decreased reactive oxygen species (ROS) levels in pancreatic islets of diabetic animals. Probucol treatment preserved the β-cell mass, the insulin content, and glucose-stimulated insulin secretion, leading to improvement of glucose tolerance

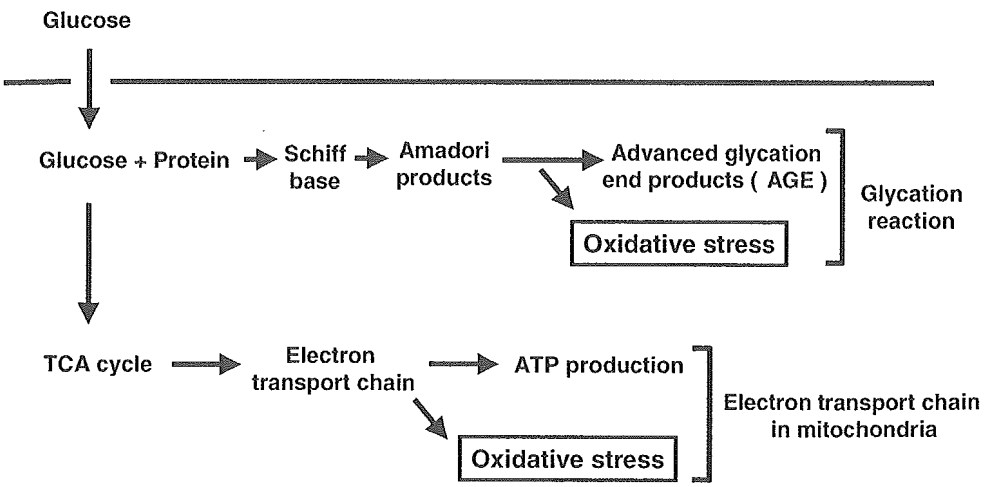

Fig. 1 Mechanisms contributing to the increase of oxidative stress under diabetic conditions

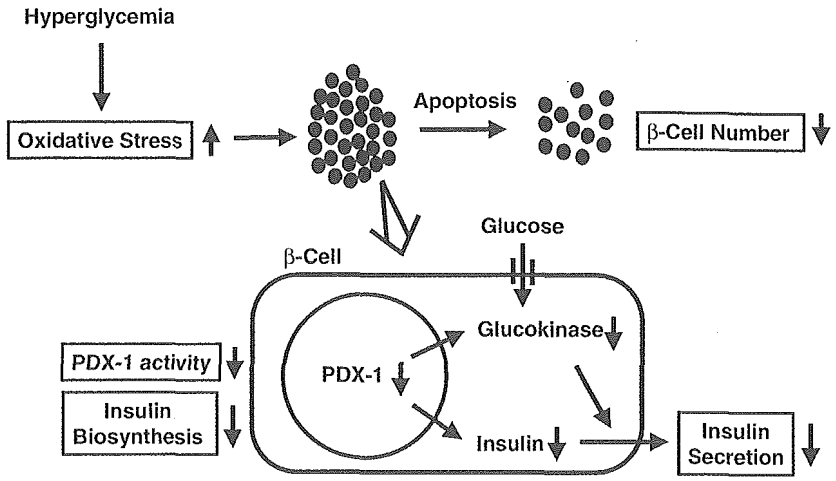

Fig. 2 Involvement of oxidative stress in pancreatic β-cell dysfunction

[13]. These data suggest a potential use for antioxidants in diabetes therapy and provides further support for the implication of oxidative stress in the β-cell glucose toxicity found in diabetes (Fig. 2).

The JNK pathway and pancreatic β-cell dysfunction

Several signal transduction pathways, including the c-Jun N-terminal kinase (JNK) [also known as stress-activated protein kinase (SAPK)] [62–65], p38 mitogen-activated protein kinase (p38 MAPK), and protein kinase C (PKC) cascades are activated by oxidative stress in various cell types, including pancreatic β-cells. It has been reported that activation of JNK is involved in the oxidative stress-induced reduction of insulin gene expression and that suppression of the JNK pathway can protect β-cells from oxidative stress [66]. When isolated rat islets were exposed to oxidative stress, the JNK, p38 MAPK, and PKC pathways were activated preceding the decrease in insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type JNK1 (DN-JNK), but neither the p38 MAPK inhibitor SB203580 nor the PKC inhibitor GF109203X, protected insulin gene expression and secretion from oxidative stress. Moreover, wild type JNK1 (WT-JNK) overexpression suppressed both insulin gene expression and secretion [66]. These results were correlated with changes in the binding of the 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 [66]. Thus, it is likely that JNK-mediated suppression of PDX-1 DNA binding activity accounts for some of this suppression of insulin gene transcription and β-cell function, which fits with the phenomenon that PDX-1 expression and DNA binding activity is decreased in association with the reduction of insulin gene transcription seen after chronic exposure to a high glucose concentration. Taken together, it is likely that activation of the JNK pathway leads to decreased PDX-1 activity and subsequent suppression of insulin gene transcription in the diabetic state (Fig. 3). Furthermore, as a potential mechanism for JNK-mediated PDX-1 inactivation, it has recently been reported that PDX-1 is translocated from the nucleus to the cytoplasm in response to oxidative stress. When β-cell-derived HIT cells were subjected to oxidative stress, both the endogenously expressed PDX-1 and the exogenously introduced green fluorescent protein (GFP) tagged PDX-1 moved from the nuclei to the cytoplasm [67] (Fig. 3). Addition of DN-JNK inhibited this oxidative stress-induced PDX-1 translocation, suggesting an essential role for JNK in mediating this phenomenon. Whereas the nuclear localization signal in PDX-1 was not affected by oxidative stress, leptomycin B, a specific inhibitor of the classic leucine-rich nuclear export signal, inhibited the nuclear-cytoplasmic translocation of PDX-1 induced by oxidative stress. Indeed, we have identified a nuclear export signal at position 82–94 of the mouse PDX-1 protein [67].

To examine whether DN-JNK can protect β-cells from the toxic effects of hyperglycemia and to explore its potential therapeutic application for islet transplantation, transplantation into diabetic mice was performed [66]. Isolated rat islets were infected with an adenoviral vector expressing dominant-negative JNK (Ad-DN-JNK) or Ad-GFP and cultured for 2 days, then 500 islets were transplanted under the kidney capsules of STZ-induced diabetic Swiss nude mice. Blood glucose levels were not significantly decreased by transplantation of islets infected with Ad-GFP, which was probably due to the toxic effects of hyperglycemia upon a marginal islet number, but were markedly decreased by Ad-DN-JNK. Four weeks after transplantation of islets infected with Ad-GFP, the insulin mRNA levels in the islet grafts were clearly decreased compared to those seen before transplantation, but were relatively well preserved by DN-JNK overexpression [66]. These results suggest that DN-JNK can protect β-cells from some of the toxic effects of hyperglycemia during the transplant period, providing new insight into the mechanism by which oxidative stress suppresses insulin gene transcription in β-cells. Also, the finding that this adverse outcome can be prevented by DN-JNK overexpression suggests that the β-cell JNK pathway could become a new therapeutic target for diabetes. It has been reported that β-cell destruction by

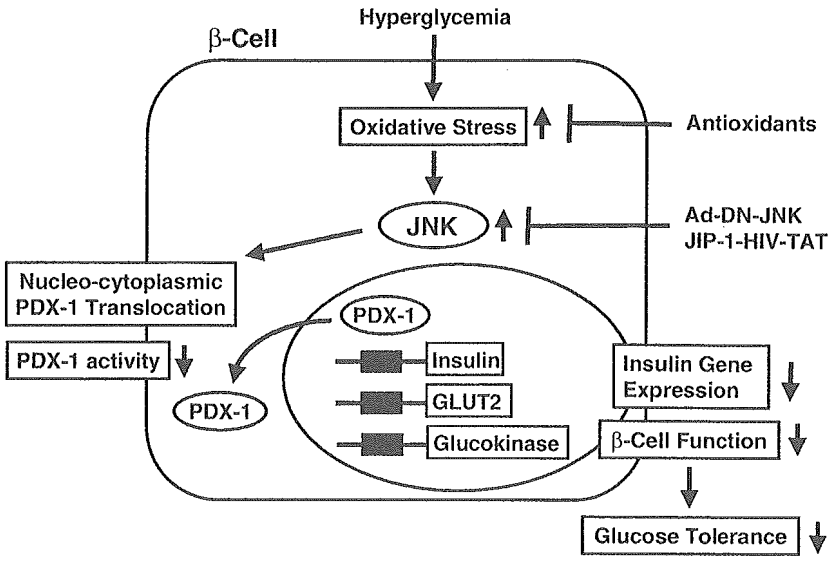

Fig. 3 Involvement of the JNK pathway in pancreatic β-cell dysfunction

cytokines such as interleukin-1β (IL-1β) [68–70] can be prevented by inhibition of the JNK pathway [71–74], implying that JNK plays a role in the autoimmune β-cell destruction observed in early stages of type 1 diabetes. It has also been reported that levels of 8-OHdG, a marker of oxidative stress, are increased in the blood of type 2 diabetic patients as well as in the islets of type 2 diabetic animal models [8, 12, 13] and that activation of JNK by oxidative stress in islets actually reduces PDX-1 DNA binding activity and insulin gene transcription [66]. In addition, the significance of JNK in the development of diabetes has also been shown in the results of a genetic analysis in humans; while islet-brain-1 (IB1), the human and rat homolog of mouse JNK-interacting protein-1 (JIP-1) [75, 76], is known to selectively inhibit the JNK signaling [74], it was reported that a missense mutation within the IB1-encoding *MAPKIP1* gene (S59N) is associated with late onset type 2 diabetes [77]. Thus, we propose that JNK is involved in the deterioration of β-cell function in both type 2 diabetes and the early stage of type 1 diabetes.

Endoplasmic reticulum stress and insulin resistance

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. Under stressful conditions such as an increase in the level of misfolded proteins, the chaperones become overloaded and the ER fails to fold and export newly synthesized proteins, leading to ER stress [78–82]. Once ER stress is provoked in the cells, various pathways are activated (Fig. 4). It has been previously reported that ER stress is involved in pancreatic β-cell apoptosis [83–87]. In Akita mice with a spontaneous mutation in the insulin 2 gene, the misfolded insulin increases ER stress, leading to decrease of β-cell mass. It has also been reported that ER overload in β-cells causes ER stress and leads to apoptosis through CHOP induction [87]. Therefore, it is likely that once ER stress is increased by appropriate stimuli, ER stress triggers apoptosis in β-cells and thus ER stress is involved in the progression of β-cell dysfunction and/or death found in diabetes.

To examine whether ER stress is increased in the liver under diabetic conditions, the ER stress level in the liver of 10-week-old obese diabetic C57BL/KsJ-*db/db* mice was evaluated. The expression levels of the immunoglobulin binding protein (Bip) and Lys-Asp-Glu-Leu (KDEL), both of which are ER stress markers, were much higher in the obese diabetic mice compared to 10-week-old non-diabetic C57BL6 mice, indicating that ER stress is actually increased under diabetic conditions [88]. 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 (Fig. 4). Therefore, phosphorylation status of PERK and eIF2α is a key indicator of the presence of ER stress [89–91]. PERK and eIF2α phosphor-

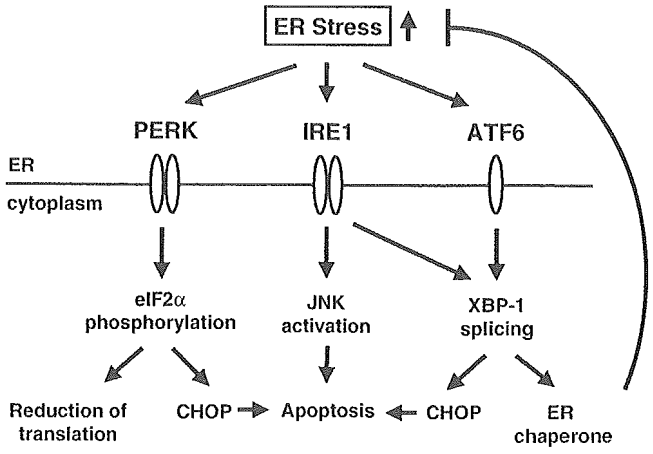

Fig. 4 ER stress signaling

ylation were increased in the liver of obese mice compared with lean controls. It is known that JNK activity is increased by ER stress (Fig. 4) [92]. Indeed, total JNK activity is also dramatically elevated in the obese mice [93]. When Fao liver cells were treated with tunicamycin or thapsigargin, both of which induce ER stress, 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 when mediated by JNK, reduces insulin receptor signaling. Indeed, pretreatment of Fao cells with tunicamycin produced a significant increase in serine phosphorylation of IRS-1. Tunicamycin pretreatment also suppressed insulin-induced Akt phosphorylation (Fig. 5) [93]. Furthermore, inhibition of JNK activity with the synthetic inhibitor SP600125 reversed the ER stress-induced serine phosphorylation of IRS-1. Pretreatment of Fao cells with a highly specific inhibitory peptide derived from the JNK-binding protein, JIP, also completely preserved insulin receptor signaling in cells exposed to tunicamycin. Similar results were obtained with the synthetic JNK inhibitor, SP600125. These results indicate that ER stress promotes a JNK-dependent serine phosphorylation of IRS-1, which in turn inhibits insulin receptor signaling (Fig. 5) [93].

Oxygen-regulated protein 150 (ORP150), a molecular chaperone found in the ER, has been shown to protect cells from ER stress [94, 95]. To examine the role of ER stress in insulin resistance in vivo, ORP150-expressing adenovirus (Ad-ORP) and a GFP-expressing control adenovirus (Ad-GFP) were delivered to 8-week-old C57BL/KsJ-*db/db* obese diabetic mice through the cervical vein. An approximately three-fold increase in ORP150 expression was seen in the liver, but not in other tissues such as muscle and adipose tissue, upon adenovirus injection. In addition, the 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 acting to decrease ER stress in the liver. There was no difference in body weight and food intake between the Ad-ORP treated and the Ad-GFP treated *db/db* mice. When C57BL/KsJ-*db/db* mice were treated with Ad-ORP, their nonfasting blood glucose levels were markedly reduced, whereas no such effect was observed in the Ad-GFP treated mice. The fasting blood glucose concentrations were also significantly lower in Ad-ORP treated mice compared to Ad-GFP treated mice, although there was no difference in plasma insulin concentrations between the two groups. To examine the effects of ORP150 overexpression in the liver on insulin resistance, intraperitoneal insulin tolerance tests were performed. The hypoglycemic response to insulin was larger in the Ad-ORP treated C57BL/KsJ-*db/db* mice compared to the Ad GFP treated mice. To investigate this point further, euglycemic hyperinsulinemic clamp tests were performed. 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) in Ad-ORP treated mice was also evaluated using tracer methods. The HGP was significantly lower in the Ad-ORP treated mice compared to the Ad-GFP treated mice. These results indicate that the reduction of insulin resistance and amelioration of glucose tolerance by Ad-ORP overexpression are mainly due to the suppression of the HGP (Fig. 5) [88].

To examine the molecular mechanism involved in the alteration of insulin action by ER stress, the phosphorylation state of IRS-1 and Akt, which are key molecules for insulin signaling, was evaluated in the liver. IRS-1 tyrosine phosphorylation was markedly increased in the Ad-ORP treated C57BL/KsJ-*db/db* mice compared to the Ad-GFP treated mice. Concomitantly, an increase in Akt serine 473 phosphorylation was observed in the Ad-ORP treated C57BL/KsJ-*db/db* mice compared to the Ad-GFP treated mice (Fig. 5). Expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), key gluconeogenic enzymes regulated by insulin signaling, were markedly decreased by Ad-ORP treatment in C57BL/KsJ-*db/db* mice. These results indicate that reduction of ER stress enhances insulin signaling, which leads to a decrease in gluconeogenesis and an amelioration of glucose tolerance [88]. Taken together, ORP150 overexpression decreased insulin resistance and markedly improved glycemic control in diabetic model animals (Fig. 5).

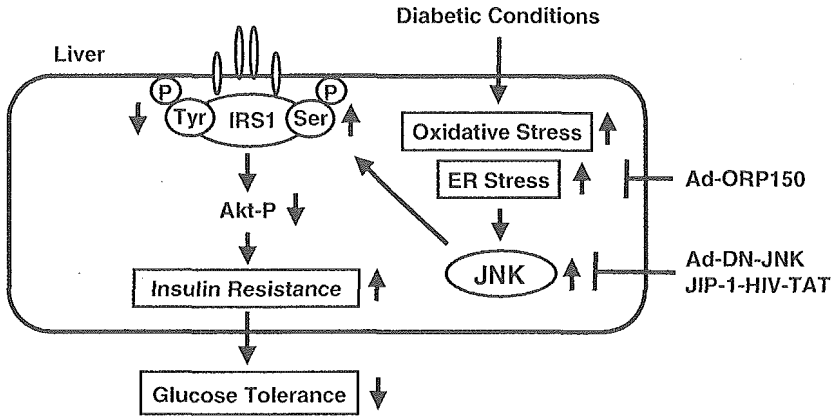

Fig. 5 Involvement of ER stress in insulin resistance

It has been reported that mice deficient in X-box binding protein-1 (XBP-1), a transcription factor that modulates the ER stress response, develop insulin resistance [93]. The spliced form of XBP-1 is a key factor in the regulation of ER stress via transcriptional regulation of various genes, including molecular chaperones (Fig. 4). In mouse embryo fibroblasts (MEFs) derived from *XBP-1*$^{-/-}$ mice, tunicamycin treatment resulted in increase of PERK phosphorylation. In these cells, there was also a marked activation of JNK in response to ER stress. When spliced XBP-1 expression was induced, there was a dramatic reduction in both PERK phosphorylation and JNK activation after tunicamycin treatment, indicating that *XBP-1*$^{-/-}$ cells are vulnerable to ER stress. Thus, it is likely that alteration of the level of spliced XBP-1 protein results in alterations in the ER stress response. Furthermore, tunicamycin-induced IRS-1 serine phosphorylation was significantly reduced in fibroblasts exogenously expressing spliced XBP-1. The extent of IRS-1 tyrosine phosphorylation was significantly higher in cells overexpressing spliced XBP-1. In contrast, IRS-1 serine phosphorylation was strongly induced in *XBP-1*$^{-/-}$ MEFs compared with *XBP-1*$^{+/+}$ controls even at low doses of tunicamycin treatment. Also, the amount of IRS-1 tyrosine phosphorylation was significantly decreased in tunicamycin treated *XBP-1*$^{-/-}$ cells compared with tunicamycintreated wild-type controls [93].

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 [93]. *XBP-1*$^{+/-}$ mice fed a high fat diet developed continuous and progressive hyperinsulinemia. Blood glucose levels were also increased in the *XBP-1*$^{+/-}$ mice fed a high fat diet. During insulin tolerance tests, the hypoglycemic response to insulin was also significantly lower in *XBP-1*$^{+/-}$ mice compared with their *XBP-1*$^{+/+}$ littermates [93]. PERK phosphorylation was increased in the liver of obese *XBP-1*$^{+/-}$ mice compared with wild type controls fed a high fat diet. There was also a significant increase in JNK activity in *XBP-1*$^{+/-}$ mice compared with wild type controls. Serine 307 phosphorylation of IRS-1 was consistently increased in the *XBP-1*$^{+/-}$ mice compared with wild type controls. There was no detectable difference in any of the insulin receptor signaling components in the liver and adipose tissues of the mice of either genotype taking a regular diet. However, after treatment with the high fat diet, the major components of insulin receptor signaling in the liver, including IRS-1 tyrosine phosphorylation and Akt serine phosphorylation, were all decreased in the *XBP-1*$^{+/-}$ mice compared with the wild type controls. A similar suppression of insulin receptor signaling was also observed in the adipose tissues of the *XBP-1*$^{+/-}$ mice compared with the *XBP-1*$^{+/+}$ mice [93]. Taken together, these results show that induction of ER stress leads to a suppression of insulin receptor signaling in intact cells via IRE-1α-dependent activation of the JNK pathway. Furthermore, deletion of an *XBP-1* allele in mice leads to systemic insulin resistance and type 2 diabetes. Thus, ER stress is involved in the progression of insulin resistance and thus could be a potential therapeutic target for diabetes (Fig. 5).

The JNK pathway and insulin resistance

The JNK pathway is known to be activated under diabetic conditions and is possibly involved in the progression of insulin resistance. The effects of modulation of the JNK pathway in the liver on insulin resistance and glucose tolerance have been studied by Nakatani et al. [96]. Overexpression of Ad-DN-JNK in the liver of obese diabetic mice dramatically improved their insulin resistance and markedly decreased their blood glucose levels. When C57BL/KsJ-*db/db* mice were treated with Ad-DN-JNK, their nonfasting blood glucose levels were markedly reduced, whereas no such effect was observed in Ad-GFP treated mice. Intraperitoneal insulin tolerance tests revealed that the hypoglycemic response to insulin was larger in the Ad-DN-JNK treated C57BL/KsJ-*db/db* mice compared to the Ad-GFP treated mice. To further investigate this point, the euglycemic hyperinsulinemic clamp test was performed. The 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 C57BL/KsJ-*db/db* mice. Furthermore, HGP was significantly lower in Ad-DN-JNK treated mice. In contrast, there was no difference in the glucose disappearance rate between these two groups [96]. 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 (Fig. 5).

It has been reported that serine phosphorylation of IRS-1 inhibits the insulin-stimulated tyrosine phosphorylation of IRS-1, leading to an increase in insulin resistance [97, 98]. The level of IRS-1 serine 307 phosphorylation was markedly decreased in the Ad-DN-JNK treated mice. An increase in IRS-1 tyrosine phosphorylation was also observed in Ad-DN-JNK treated mice compared to the control mice. Reduction of Akt serine 473 phosphorylation was also observed in Ad-DN-JNK treated C57BL/KsJ-*db/db* mice [96]. Therefore, an increase in IRS-1 serine phosphorylation may be closely associated with the development of insulin resistance induced by JNK overexpression. The expression levels of PEPCK and G6Pase, key gluconeogenic enzymes regulated by insulin signaling, were markedly decreased by Ad-DN-JNK treatment in C57BL/KsJ-*db/db* mice. These results indicate that suppression of the JNK pathway enhances insulin signaling, which leads to a decrease in gluconeogenesis and an amelioration of glucose tolerance. Similar effects were observed in mice suffering diabetes induced by feeding a high-fat/high-sucrose diet. Conversely, expression of wild type JNK in the liver of normal mice decreased insulin sensitivity [96]. Taken together, these findings suggest that suppression of the JNK pathway in the liver exerts a beneficial effect on insulin resistance status and glucose tolerance in both genetic and dietary models of diabetes (Fig. 5).

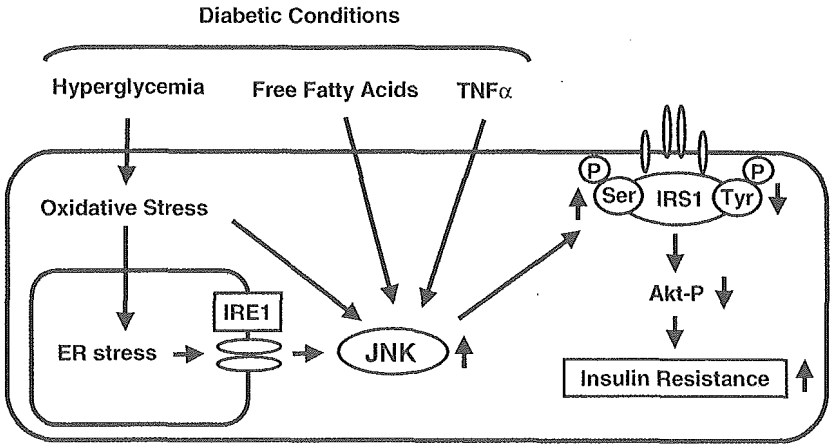

Fig. 6 Involvement of the JNK pathway in insulin resistance

It has also recently been reported that JNK activity is abnormally elevated in the liver, muscle and adipose tissues of obese type 2 diabetic mice and that insulin resistance is substantially reduced in mice homozygous for a targeted mutation in the *JNK1* gene (JNK-KO mice) [97]. 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, the blood glucose levels of the obese JNK-KO mice were significantly lower than those of the obese wild type mice. In addition, the serum insulin levels of the obese JNK-KO mice were significantly lower compared to those of the obese wild type mice. Intraperitoneal insulin tolerance tests showed that the hypoglycemic response to insulin in obese wild type mice was lower than that of obese JNK-KO mice. Also, intraperitoneal glucose tolerance tests revealed a greater 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 into genetically obese mice (*ob/ob*) [97]. Blood glucose levels in the *ob/ob*-JNK-KO mice were lower than 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 to obesity, hyperglycemia and hyperinsulinemia in both genetic and dietary models of diabetes. Taken together, obesity-induced type 2 diabetes is associated with activation of the JNK pathway, and the absence of JNK activity results in substantial protection from obesity-induced insulin resistance. These results strongly suggest that JNK plays a crucial role in the progression of insulin resistance found in type 2 diabetes. It is should be noted 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 [97]. 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. In addition, 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, 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 suppression of the JNK pathway may at least in part counterbalance the deleterious effects of several factors such as oxidative stress, FFAs, and TNF-α (Fig. 6).

Protein transduction domains such as those of the TAT protein of the 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 efficiently deliver various proteins and peptides 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 [99–104]. It has recently been reported that the cell-permeable JNK inhibitory peptide (amino acid sequence GRKKRRQRRRPPRPKRPTTLNL FPQVPRSQDT) is effective for the treatment of diabetes. This peptide is derived from the JNK binding domain of the JNK-interacting protein-1 (JIP-1), also known as islet brain-1 (IB-1), and has been reported to function as a

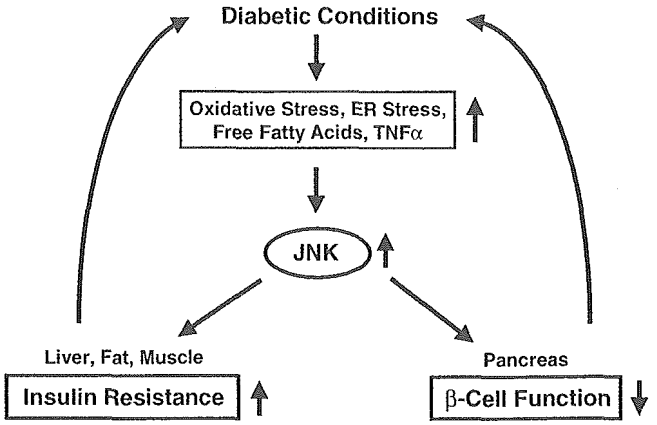

Fig. 7 *Role of the JNK pathway in diabetes*

dominant inhibitor of the JNK pathway [74]. 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 (RPKRPTTLNLFPQVP RSQDT) was covalently linked to a 10-amino acid carrier peptide derived from the HIV-TAT sequence (GRKKRRQ RRR), then to monitor peptide delivery this JIP-1-HIV-TAT peptide was further conjugated to fluorescein isothiocyanate (FITC). First, to examine the effectiveness of the JNK inhibitory peptide in vivo, C57BL/KsJ-*db/db* obese diabetic mice were intraperitoneally injected with the JIP-1-HIV-TAT-FITC peptide. The FITC-conjugated peptide showed fluorescent signals in the insulin target organs (liver, fat, muscle) and in the insulin secreting tissue (pancreatic islets). In various tissues (liver, fat, and muscle), JNK activity was also suppressed by JIP-1-HIV-TAT-FITC in a dose-dependent manner [105].

To investigate whether suppression of the JNK pathway exerts a beneficial effect on diabetes, C57BL/KsJ-*db/db* mice were treated with intraperitoneal injections of JIP-1-HIV-TAT-FITC or a scramble peptide as a control. There was no difference in body weight and food intake between the JIP-1-HIV-TAT-FITC treated and control mice. The non-fasting blood glucose levels in mice treated with JIP-1-HIV-TAT-FITC were significantly decreased compared with those of the untreated or the scramble peptide treated mice. Also, glucose tolerance tests showed that the glucose tolerance of the JIP-1-HIV-TAT-FITC treated mice was significantly ameliorated compared that of the untreated or the scramble peptide treated mice [105]. These data indicate that the JNK pathway is involved in the exacerbation of diabetes and that suppression of the JNK pathway may be a therapeutic target for diabetes (Fig. 7). To investigate the possible effects of the JNK inhibitory peptide on insulin action, insulin tolerance tests were performed. The reduction of blood glucose levels in response to insulin injection was much larger in the JIP-HIV-TAT-FITC treated mice compared to the untreated mice, indicating that the peptide treatment improves insulin sensitivity. In contrast, no such effect was observed when non-diabetic C57BL6 mice were treated with JIP-1-HIV-TAT-FITC. These results imply that the JNK pathway is activated under diabetic conditions and thus the JNK inhibitory peptide exerts beneficial effects on insulin action and glucose tolerance. To further investigate the effect of the peptide on insulin resistance, euglycemic hyperinsulinemic clamp tests were performed. The steady-state GIR in JIP-1-HIV-TAT-FITC treated mice was significantly higher than that of the untreated mice, indicating that JIP-1-HIV-TAT-FITC reduces insulin resistance in C57BL/KsJ-*db/db* mice. The endogenous HGP and glucose disappearance rate in the JNK inhibitory peptide treated mice were also evaluated. It should be noted that the glucose disappearance rate reflects glucose utilization in the peripheral tissues. In the JIP-1-HIV-TAT-FITC treated mice, HGP was significantly lower than in the untreated mice, and the glucose disappearance rate was significantly higher in the treated than in the untreated mice [105]. These results indicate that JIP-1-HIV-TAT-FITC treatment reduces insulin resistance through decreasing HGP and increasing the glucose disappearance rate. These data provide strong evidence that JNK is indeed a crucial component of the biochemical pathway responsible for insulin resistance in vivo. In addition, the insulin mRNA levels and insulin content were significantly higher in the peptide treated mice. Thus, we assume that the JNK inhibitory peptide exerted some beneficial effects on the pancreatic islets. To explore the molecular mechanism of how JIP-1-HIV-TAT-FITC treatment improves insulin sensitivity and ameliorates glucose tolerance, IRS-1 serine 307 phosphorylation was evaluated in various insulin target tissues (liver, fat, and muscle) of JIP-1-HIV-TAT-FITC treated mice. IRS-1 serine 307 phosphorylation was decreased in the JIP-1-HIV-TAT-FITC treated mice compared to the control mice. An increase in IRS-1 tyrosine phosphorylation was observed in the peptide treated mice compared to the control mice. Concomitantly, an increase of Akt serine 473 and threonine 308 phosphorylation, both of which are known to be important for activation of the Akt pathway, was observed in the JIP-1-HIV-TAT-FITC treated mice [105]. In conclusion, the cell-permeable JNK inhibitory peptide, JIP-1-HIV-TAT-FITC, 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. 7).

Conclusions

Oxidative stress and ER stress are induced in various tissues under diabetic conditions, leading to activation of the JNK pathway. Activation of this pathway is involved in the progression of insulin resistance as well as the deterioration of pancreatic β-cell function. Indeed, suppression of the JNK pathway in obese diabetic mice markedly improves insulin resistance and β-cell function, leading to an amelioration of glucose tolerance. Taken together, these observations suggest that the JNK pathway plays a crucial role in the progression of insulin resistance as well as in β-cell dysfunction and thus could be a potential therapeutic target for diabetes.

References

1. Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414:799–806
2. Shulman GI (2000) Cellular mechanisms of insulin resistance. J Clin Invest 106:171–176
3. Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A (2001) β-Cell adaptation and decompensation during the progression of diabetes. Diabetes 50:S154–S159
4. Poitout V, Robertson RP (2002) Minireview: secondary beta-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity. Endocrinology 143:339–342

5. Sharma A, Olson LK, Robertson RP, Stein R (1995) The reduction of insulin gene transcription in HIT-T15β cells chronically exposed to high glucose concentration is associated with loss of RIPE3b1 and STF-1 transcription factor expression. Mol Endocrinol 9:1127–1134
6. Moran A, Zhang H-J, Olson LK, Harmon JS, Poitout V, Robertson RP (1997) Differentiation of glucose toxicity from beta cell exhaustion during the evolution of defective insulin gene expression in the pancreatic islet cell line, HIT-T15. J Clin Invest 99:534–539
7. Jonas J-C, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC (1999) Chronic hyperglycemia triggers loss of pancreatic β cell differentiation in an animal model of diabetes. J Biol Chem 274:14112–14121
8. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T (1996) Oxidative damage to DNA in diabetes mellitus. Lancet 347:444–445
9. Baynes JW, Thorpe SR (1999) Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 48:1–9
10. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404:787–790
11. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820
12. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y (1999) Hyperglycemia causes oxidative stress in pancreatic β-cells of GK rats, a model of type 2 diabetes. Diabetes 48:927–932
13. Gorogawa S, Kajimoto Y, Umayahara Y, Kaneto H, Watada H, Kuroda A, Kawamori D, Yasuda T, Matsuhisa M, Yamasaki Y, Hori M (2002) Probucol preserves pancreatic β-cell function through reduction of oxidative stress in type 2 diabetes. Diabetes Res Clin Pract 57:1–10
14. Kaneto H, Fujii J, Myint T, Islam KN, Miyazawa N, Suzuki K, Kawasaki Y, Nakamura M, Tatsumi H, Yamasaki Y, Taniguchi N (1996) Reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress through the glycation reaction. Biochem J 320:855–863
15. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, Fujitani Y, Kamada T, Kawamori R, Yamasaki Y (1997) Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J Clin Invest 99:144–150
16. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, Hori M (1999) Beneficial effects of antioxidants for diabetes: possible protection of pancreatic β-cells against glucose toxicity. Diabetes 48:2398–2406
17. Tanaka Y, Gleason CE, Tran POT, Harmon JS, Robertson RP (1996) Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. Proc Natl Acad Sci USA 96:10857–10862
18. Kaneto H, Kajimoto Y, Fujitani Y, Matsuoka T, Sakamoto K, Matsuhisa M, Yamasaki Y, Hori M (1999) Oxidative stress induces p21 expression in pancreatic islet cells: possible implication in β-cell dysfunction. Diabetologia 42:1093–1097
19. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y, Hori M (1999) Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells. Diabetologia 42:1417–1424
20. Maechler P, Jornot L, Wollheim CB (1999) Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. J Biol Chem 74:27905–27913
21. Kaneto H, Xu G, Song K-H, Suzuma K, Bonner-Weir S, Sharma A, Weir GC (2001) Activation of the hexosamine pathway leads to deterioration of pancreatic β-cell function by provoking oxidative stress. J Biol Chem 276:31099–31104
22. Tanaka Y, Tran POT, Harmon J, Robertson RP (2002) A role of glutathione peroxidase in protecting pancreatic β cells against oxidative stress in a model of glucose toxicity. Proc Natl Acad Sci USA 99:12363–12368
23. Sakai K, Matsumoto K, Nishikawa T, Suefuji M, Nakamura K, Hirashima Y, Kawashima J, Shirotani T, Ichinose K, Brownlee M, Araki E (2003) Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic β-cells. Biochem Biophys Res Commun 300:216–222
24. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H (2003) Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 52:581–587
25. Robertson RP (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem 279:42351–42354
26. Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 52:1–8
27. Lenzen S, Drinkgern J, Tiedge M (1996) Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med 20:463–466
28. Tiedge M, Lortz S, Drinkgern J, Lenzen S (1997) Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46:1733–1742
29. Ohlsson H, Karlsson K, Edlund T (1993) IPF1, a homeodomain-containing-transactivator of the insulin gene. EMBO J 12:4251–4259
30. Leonard J, Peers B, Johnson T, Ferreri K, Lee S, Montminy MR (1993) Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol 7:1275–1283
31. Miller CP, McGehee RE, Habener JF (1994) IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. EMBO J 13:1145–1156
32. Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 37:606–609
33. Offield MF, Jetton TL, Labosky P, Ray M, Stein R, Magnuson M, Hogan BLM, Wright CVE (1996) PDX-1 is required for pancreas outgrowth and differentiation of the rostral duodenum. Development 122:983–985
34. Ahlgren U, Jonsson J, Edlund H (1996) The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development 122:1409–1416
35. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human *IPF1* gene coding sequence. Nat Genet 15:106–110
36. Kaneto H, Miyagawa J, Kajimoto Y, Yamamoto K, Watada H, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y, Higashiyama S, Taniguchi N (1997) Expression of heparin-binding epidermal growth factor-like growth factor during pancreas development: a potential role of PDX-1 in transcriptional activation. J Biol Chem 272:29137–29143
37. Sander M, German MS (1997) The beta cell transcription factors and development of the pancreas. J Mol Med 75:327–340
38. Dutta S, Bonner-Weir S, Montminy M, Wright C (1998) Regulatory factor linked to late-onset diabetes? Nature 392:560
39. Stoffers DA, Heller RS, Miller CP, Habener JF (1999) Developmental expression of the homeodomain protein IDX-1 mice transgenic for an *IDX-1* promoter/LacZ transcriptional reporter. Endocrinology 140:5374–5381
40. Holland AM, Hale MA, Kagami H, Hammer RE, MacDonald RJ (2002) Experimental control of pancreatic development and maintenance. Proc Natl Acad Sci USA 99:12236–12241

41. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song K-H, Sharma A, O'Neil JJ (2000) In vitro cultivation of human islets expanded ductal tissue. Proc Natl Acad Sci USA 97:7999–8004
42. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seijffers R, Kopolovic J, Kaiser N, Karasik A (2000) Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 6:568–572
43. Kojima H, Nakamura T, Fujita Y, Kishi A, Fujimiya M, Yamada S, Kudo M, Nishio Y, Maegawa H, Haneda M, Yasuda H, Kojima I, Seno M, Wong NCW, Kikkawa R, Kashiwagi A (2002) Combined expression of pancreatic duodenal homeobox 1 and islet factor 1 induces immature enterocytes to produce insulin. Diabetes 51:1398–1408
44. Yoshida S, Kajimoto Y, Yasuda T, Watada H, Fujitani Y, Kosaka H, Gotow T, Miyatsuka T, Umayahara Y, Yamasaki Y, Hori M (2002) PDX-1 induces differentiation of intestinal epithelioid IEC-6 into insulin-producing cells. Diabetes 51:2505–2513
45. Ber I, Shternhall K, Perl S, Ohanuna Z, Goldberg I, Barshack I, Benvenisti-Zarum L, Meivar-Levy I, Ferber S (2003) Functional, persistent, and extended liver to pancreas transdifferentiation. J Biol Chem 22:31950–31957
46. Miyatsuka T, Kaneto H, Kajimoto Y, Hirota S, Arakawa Y, Fujitani Y, Umayahara Y, Watada H, Yamasaki Y, Magnuson MA, Miyazaki J, Hori M (2003) Ectopically expressed PDX-1 in liver initiates endocrine and exocrine pancreas differentiation but causes dysmorphogenesis. Biochem Biophys Res Commun 310:1017–1025
47. Noguchi H, Kaneto H, Weir GC, Bonner-Weir S (2003) PDX-1 protein containing its own Antennapedia-like protein transduction domain can transduce pancreatic duct and islet cells. Diabetes 52:1732–1737
48. Moritoh Y, Yamato E, Yasui Y, Miyazaki S, Miyazaki J (2003) Analysis of insulin-producing cells during in vitro differentiation from feeder-free embryonic stem cells. Diabetes 52:1163–1168
49. Taniguchi H, Yamato E, Tashiro F, Ikegami H, Ogihara T, Miyazaki J (2003) β-Cell neogenesis induced by adenovirus-mediated gene delivery of transcription factor pdx-1 into mouse pancreas. Gene Ther 10:15–23
50. Tang D-Q, Cao L-Z, Burkhardt BR, Xia C-Q, Litherland SA, Atkinson MA, Yang L-J (2004) In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. Diabetes 53:1721–1732
51. Miyazaki S, Yamato E, Miyazaki J (2004) Regulated expression of pdx-1 promotes in vitro differentiation of insulin-producing cells from embryonic stem cells. Diabetes 53:1030–1037
52. Petersen HV, Serup P, Leonard J, Michelsen BK, Madsen OD (1994) Transcriptional regulation of the human insulin gene is dependent on the homeodomain protein STF1/IPF1 acting through the CT boxes. Proc Natl Acad Sci USA 91:10465–10469
53. Peers B, Leonard J, Sharma S, Teitelman G, Montminy MR (1994) Insulin expression in pancreatic islet cells relies on cooperative interactions between the helix loop helix factor E47 and the homeobox factor STF-1. Mol Endocrinol 8:1798–1806
54. Waeber G, Thompson N, Nicod P, Bonny C (1996) Transcriptional activation of the *GLUT2* gene by the IPF-1/STF-1/IDX-1 homeobox factor. Mol Endocrinol 10:1327–1334
55. Watada H, Kajimoto Y, Umayahara Y, Matsuoka T, Kaneto H, Fujitani Y, Kamada T, Kawamori R, Yamasaki Y (1996) The human glucokinase gene β-cell-type promoter: an essential role of insulin promoter factor 1 (IPF1)/PDX-1 in its activation in HIT-T15 cells. Diabetes 45:1478–1488
56. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H (1998) β-cell-specific inactivation of the mouse *Ipf1/Pdx1* gene results in loss of the β-cell phenotype and maturity onset diabetes. Genes Dev 12:1763–1768
57. Wang H, Maechler P, Ritz-Laser B, Hagenfeldt KA, Ishihara H, Philippe J, Wollheim CB (2001) Pdx1 level defines pancreatic gene expression pattern and cell lineage differentiation. J Biol Chem 276:25279–25286
58. Brissova M, Shiota M, Nicholson WE, Gannon M, Knobel SM, Piston DW, Wright CV, Powers AC (2002) Reduction in pancreatic transcription factor PDX-1 impairs glucose-stimulated insulin secretion. J Biol Chem 277:11225–11232
59. Chakrabarti SK, James JC, Mirmira RG (2002) Quantitative assessment of gene targeting in vitro and in vivo by the pancreatic transcription factor, pdx1: importance of chromatin structure in directing promoter binding. J Biol Chem 277:13286–13293
60. Kulkarni RN, Jhala US, Winnay JN, Krajewski S, Montminy M, Kahn CR (2004) *PDX-1* haploinsufficiency limits the compensatory islet hyperplasia that occurs in response to insulin resistance. J Clin Invest 114:828–836
61. Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type-II diabetes mellitus (MODY4) linked to *IPF1*. Nat Genet 17:138–139
62. Hibi M, Lin A, Karin M (1993) Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev 7:2135–2148
63. Derijard B, Hibi M, Wu I-H, Barrett T, Su B, Deng T, Karin M, Davis RJ (1994) JNK1: a protein kianse stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell 76:1025–1037
64. Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. Cell 103:239–252
65. Chang L, Karin M (2001) Mammalian MAP kinase signalling cascades. Nature 410:37–40
66. Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC (2002) Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. J Biol Chem 277:30010–30018
67. Kawamori D, Kajimoto Y, Kaneto H, Umayahara Y, Fujitani Y, Miyatsuka T, Watada H, Leibiger IB, Yamasaki Y, Hori M (2003) Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun N-terminal kinase. Diabetes 52:2896–2904
68. Corbett JA, Wang JL, Sweetland MA, Lancaster JR Jr, McDaniel ML (1992) Interleukin 1β induces the formation of nitric oxide by β-cells purified from rodent islets of Langerhans: evidence for the β-cell as a source and site of action of nitric oxide. J Clin Invest 90:2384–2391
69. Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Nieman A, Geller DA, Pipeleers DG, Bendtzen K, Hellerstrom C (1994) Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. J Clin Invest 93:1968–1974
70. Kaneto H, Fujii J, Seo HG, Suzuki K, Matsuoka T, Nakamura N, Tatsumi H, Yamasaki Y, Kamada T, Taniguchi N (1995) Apoptotic cell death triggered by nitric oxide in pancreatic β-cells. Diabetes 44:733–738
71. Ammendrup A, Maillard A, Nielsen K, Aabenhus Andersen N, Serup P, Dragsbaek Madsen O, Mandrup-Poulsen T, Bonny C (2000) The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic β-cells. Diabetes 49:1468–1476
72. Bonny C, Oberson A, Steinmann M, Schorderet DF, Nicod P, Waeber G (2000) IB1 reduces cytokine-induced apoptosis of insulin-secreting cells. J Biol Chem 275:16466–16472
73. Mandrup-Poulsen T (2001) β-cell apoptosis: stimuli and signaling. Diabetes 50:S58–S63
74. Bonny C, Oberson A, Negri S, Sause C, Schorderet DF (2001) Cell-permeable peptide inhibitors of JNK: novel blockers of β-cell death. Diabetes 50:77–82
75. Bonny C, Nicod P, Waeber G (1998) IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. J Biol Chem 273:1843–1846

76. Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern JR, Greenberg ME, Sawyers CL, Davis RJ (1997) A cytoplasmic inhibitor of the JNK signal transduction pathway. Science 277:20010–20018
77. Waeber G, Delplanque J, Bonny C, Mooser V, Steinmann M, Widmann C, Maillard A, Miklossy J, Dina C, Hani EH, Vionnet N, Nicod P, Boutin P, Froguel P (2000) The gene *MAPK8IP1*, encoding islet-brain-1, is a candidate for type 2 diabetes. Nat Genet 24:291–295
78. Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D (1998) Cloning of mammalian Ire1 reveals diversity in the ER stress responses. EMBO J 17:5708–5717
79. Tirasophon W, Welihinda AA, Kaufman RJ (1998) A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes Dev 12:1812–1824
80. Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274
81. Aridor M, Balch WE (1999) Integration of endoplasmic reticulum signaling in health and disease. Nat Med 5:745–751
82. Ron D (2002) Translational control in the endoplasmic reticulum stress response. J Clin Invest 110:1383–1388
83. Inoue H, Tanizawa Y, Wasson J, Behn P, Kalidas K, Bernal-Mizrachi E, Mueckler M, Marshall H, Donis-Keller H, Crock P, Rogers D, Mikuni M, Kumashiro H, Higashi K, Sobue G, Oka Y, Permutt MA (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). Nat Genet 20:143–148
84. Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD, Ron D (2001) Diabetes mellitus and exocrine pancreatic dysfunction in perk−/− mice reveals a role for translational control in secretory cell survival. Mol Cell 7:1153–1163
85. Harding HP, Ron D (2002) Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes 51:S455–S461
86. Oyadomari S, Takeda K, Takiguchi M, Gotoh T, Matsumoto M, Wada I, Akira S, Araki E, Mori M (2001) Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. Proc Natl Acad Sci USA 98:10845–10850
87. Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, Mori M (2002) Targeted disruption of the *Chop* gene delays endoplasmic reticulum stress-mediated diabetes. J Clin Invest 109:525–532
88. Nakatani Y, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka T, Ozawa K, Ogawa T, Hori M, Yamasaki Y, Matsuhisa M (2005) Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. J Biol Chem 280:847–851
89. Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274
90. Shi Y, Taylor SI, Tan SL, Sonenberg N (2003) When translation meets metabolism: multiple links to diabetes. Endocr Rev 24:91–101
91. Shi Y, Vattem KM, Sood R, An J, Liang J, Stramm L, Wek RC (1998) Identification and characterization of pancreatic eukaryotic initiation factor 2α-subunit kinase, PEK, involved in translational control. Mol Cell Biol 18:7499–7509
92. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287:664–666
93. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306:457–461
94. Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T, Stern DM, Yanagi H, Kamada T (1996) Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. J Biol Chem 271:5025–5032
95. Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi M, Che YH, Ozawa K, Hori O, Nishimura H, Yamashita A, Okabe M, Yanagi H, Stern DM, Ogawa S, Tohyama M (2001) ORP150 protects against hypoxia/ischemia-induced neuronal death. Nat Med 7:317–323
96. Nakatani Y, Kaneto H, Kawamori D, Hatazaki M, Miyatsuka T, Matsuoka T, Kajimoto Y, Matsuhisa M, Yamasaki Y, Hori M (2004) Modulation of the JNK pathway in liver affects insulin resistance status. J Biol Chem 279:45803–45809
97. Hirosumi J, Tuncman G, Chang L, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. Nature 420:333–336
98. Aguirre V, Davis R, White MF (2000) The c-Jun NH_2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser^{307}. J Biol Chem 275:9047–9054
99. Schwarze SR, Ho A, Vocero-Akbani AM, Dowdy SF (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285:1569–1572
100. Elliott G, O'Hare P (1997) Intracellular trafficking and protein delivery by a herpes virus structure protein. Cell 88:223–233
101. Frankel AD, Pabo CO (1988) Cellular uptake of the tat protein from human immunodeficiency virus. Cell 55:1189–1193
102. Nagahara H, Vocero-Akbani AM, Snyder EL, Ho A, Latham DG, Lissy NA, Becker-Hapak M, Ezhevsky SA, Dowdy SF (1998) Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p-27^{Kip1} induces cell migration. Nat Med 4:1449–1452
103. Rothbard JB, Garlington S, Lin Q, Kirschberg T, Kreider E, McGrane PL, Wender PA, Khavari PA (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. Nat Med 6:1253–1257
104. Noguchi H, Matsushita M, Okitsu T, Moriwaki A, Tomizawa K, Kang S, Li ST, Kobayashi N, Matsumoto S, Tanaka K, Tanaka N, Matsui H (2004) A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. Nat Med 10:305–309
105. Kaneto H, Nakatani Y, Miyatsuka T, Kawamori D, Matsuoka T, Matsuhisa M, Kajimoto Y, Ichijo H, Yamasaki Y, Hori M (2004) Possible novel therapy for diabetes with cell-permeable JNK inhibitory peptide. Nat Med 10:1128–1132

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Oxidative Stress and Pancreatic β-Cell Dysfunction

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Oxidative stress is induced under diabetic conditions through various pathways, including the electron transport chain in mitochondria and the nonenzymatic glycosylation reaction, and is likely involved in progression of pancreatic β-cell dysfunction developing in diabetes. β-Cells are vulnerable to oxidative stress, possibly due to low levels of antioxidant enzyme expression. When oxidative stress was induced in vitro in β cells, the insulin gene promoter activity and mRNA levels were suppressed, accompanied by the reduced activity of pancreatic and duodenal homeobox factor-1 (PDX-1) (also known as IDX-1/STF-1/IPF1), an important transcription factor for the insulin gene. The suppression of oxidative stress by a potent antioxidant, *N*-acetyl-L-cysteine or probucol, led to the recovery of insulin biosynthesis and PDX-1 expression in nuclei and improved glucose tolerance in animal models for type 2 diabetes. As a possible cause of this, we recently found that PDX-1 was translocated from the nucleus to the cytoplasm in response to oxidative stress. Furthermore, the addition of a dominant-negative form of c-Jun N-terminal kinase (JNK) inhibited the oxidative stress-induced PDX-1 translocation, suggesting an essential role of JNK in mediating the phenomenon. Taken together, the oxidative stress–mediated activation of the JNK pathway leads to nucleocytoplasmic translocation of PDX-1 and thus is likely involved in the progression of β-cell dysfunction found in diabetes.

Keywords: glucose toxicity, oxidative stress, insulin, PDX-1, JNK

PANCREATIC β-CELL GLUCOSE TOXICITY AND OXIDATIVE STRESS

The development of type 2 diabetes is usually associated with a combination of β-cell dysfunction and insulin resistance. Normal β cells compensate for insulin resistance by an increasing insulin secretion or β-cell mass, whereas insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, the β-cell function further deteriorates. This β-cell dysfunction is caused at least in a part by the adverse effects of chronic hyperglycemia. This process is called β-cell glucose toxicity, which has been demonstrated in various in vivo and in vitro studies, with a key characteristic being the suppression of insulin gene transcription and secretion.[1–6] Histologically, such damaged β cells often reveal an extensive insulin degranulation, which is clinically associated with the development of type 2 diabetes.

Under diabetic conditions, reactive oxygen species (ROS) are produced in various tissues, such as nerve cells and vascular endothelial cells, and are involved in the development of diabetic complications.[7,8] There are several sources of ROS productions in cells: the electron transport chain in mitochondria,[8] the nonenzymatic glycosylation reaction,[9] and the hexosamine pathway.[10] Recently, pancreatic β cells emerged as a target of oxidative stress-mediated tissue damage (Fig. 1).[10–19] Due to the relatively low expression of antioxidant enzymes, such as catalase and glutathione peroxidase,[20] β cells are rather sensitive to the ROS attack when exposed to oxidative stress. Thus, it is likely that oxidative stress plays a major role in β-cell deterioration in type 2 diabetes.

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