Pdx1 Expression in Irs2-deficient Mouse β-Cells Is Regulated in a Strain-dependent Manner*

Received for publication, July 1, 2003
Published, JBC Papers in Press, July 16, 2003, DOI 10.1074/jbc.M307004200

We previously demonstrated that Irs2−/− mice develop diabetes due to β-cell growth failure and insulin resistance; however, glucose-induced insulin secretion was increased in islets isolated from Irs2−/− mice. Pdx-1, a transcription factor important for maintenance of the β-cell function, was recently reported to be severely reduced in Irs2−/− murine β-cells. We report herein that Pdx-1 expression, including the amount of Pdx-1 localized in the nucleus, is not down-regulated in our Irs2−/− murine β-cells with a C57BL/6 background. We have also demonstrated the expression of upstream genes of Pdx-1, such as HNF3β and HNF1α, as well as its downstream genes, including insulin, Glut2, and Nkx6.1, to be well preserved. We have further demonstrated Pdx-1 expression to also be preserved in β-cells of 30-week-old diabetic Irs2−/− mice. In addition, surprisingly, even in Irs2−/− mice on a high fat diet with markedly elevated blood glucose, exceeding 400 mg/dl, Pdx-1 expression was not reduced. Furthermore, we found Pdx-1 to be markedly decreased in certain severely diabetic Irs2−/− mice with a mixed C57BL/6J × 129sv background. We conclude that 1) Pdx-1 expression in Irs2−/− mice is regulated in a strain-dependent manner, 2) Irs2−/− mice develop diabetes associated with β-cell growth failure even when Pdx1 expression is preserved, and 3) Pdx-1 expression is preserved in severely hyperglycemic Irs2−/− mice with a C57BL/6 background on a high fat diet.

Insulin receptor substrate (IRS)1-1 and IRS-2 are two major substrates for insulin receptor tyrosine kinase and insulin-like growth factor (IGF) receptor tyrosine kinase (1–6). Irs1−/− mice have insulin-resistant skeletal muscles but do not develop diabetes because β-cells are able to undergo hyperplasia and secrete more insulin to compensate for the insulin resistance (7, 8). In contrast, Irs2−/− mice develop diabetes because β-cells failed to undergo hyperplasia in the face of hepatic insulin resistance (9–12). Whereas β-cell mass in Irs2−/− mice was reduced to 83% of that in the wild-type mice at the age of 6 weeks, β-cell mass in Irs2−/− mice was significantly reduced to 51% at 12 weeks of age (11). Insulin/insulin-like growth factor signaling through IRS and phosphoinositide 3-kinase has been thought to regulate several aspects of β-cell function (13–17). Besides the impaired β-cell proliferation in Irs2−/− mice, ablation of p70S6K, an Akt substrate, is associated with a decrease in β-cell size (18). Conversely, overexpression of a constitutively active mutant Akt1 increases β-cell mass and protects from streptozocin-induced diabetes (19, 20). However, neither the ablation of p70S6K nor β-cell-specific overexpression of Akt1 affects glucose sensing or insulin production (18, 20). In fact, we also previously demonstrated glucose-induced insulin secretion to be rather increased in Irs2−/− murine islets (11). In addition, insulin contents of Irs2−/− murine islets were comparable with those of islets from wild-type mice (11), suggesting the β-cell functions of insulin synthesis and secretion to be well preserved in Irs2−/− murine islets.

Pdx-1 is a key transcription factor involved in pancreatic development in early embryonic stages (21, 22) and is also involved in the maintenance of β-cell functions in later embryonic stages (23–25). Pdx-1 is also reported to be involved in insulin secretory function in both adult mice and cultured β-cell lines (25, 26). Pdx-1 expression reportedly lies downstream from HNF3β and HNF1α (27) and regulates insulin, Glut2, Nkx6.1, and glucokinase genes (28–33). In adult islets, Pdx-1 is required for maintenance of the β-cell phenotype, including expression of insulin and Glut2 (34). Pdx-1 is also known as a MODY (maturity onset diabetes of the young) 4 gene (35). Haploinsufficiency of the Pdx-1 gene in mice reportedly results in defects in glucose-stimulated insulin secretion (23, 25, 26). The Pdx-1 mutation identified in human MODY4 patients may serve as a dominant-negative isoform (36).

Pdx-1 was recently reported to be severely reduced in Irs2−/− murine β-cells, suggesting the possibility that IRS-2 directly regulates the expression and function of Pdx-1, thereby maintaining β-cell growth and function (37). These studies indicated...
that Pdx-1 mRNA levels in Irs2−/− islets are reduced to about 20% of those in wild-type islets, associated with HNF3β mRNA reduction, and that the protein levels are reduced identically. However, because glucose-induced insulin secretion was increased and the insulin content was well preserved in our Irs2−/− murine islets (11), we suspected that Pdx-1 is not

**Fig. 1. Expression of Pdx-1 in pancreatic islets.** a, TaqMan RT-PCR of Pdx-1 in islets from 8-week-old Irs2+/+ (n = 6) and Irs2−/− (n = 6) male mice. Total RNA was extracted from each mouse. Data are normalized to the expression of the β-actin gene (1), GAPDH (2), and cyclophillin (3). Pdx-1 mRNA levels in Irs2−/− mouse islets were unchanged when β-actin and GAPDH were used as internal standard and were increased when cyclophillin was used. N.S., difference not significant. b, Western blots of Pdx-1 in islets isolated from 8-week-old Irs2+/+ and Irs2−/− male mice. For each group, islets from three mice were pooled, and lysates (20 μg of protein) were subjected to Western blot analysis with antibodies against Pdx-1 and actin as an internal control. Pdx-1 protein was not decreased in Irs2−/− mouse islets.
down-regulated in our \textit{Irs2}^{-/-} mouse. We report herein that Pdx-1 expression, including the amount of Pdx-1 localized in the nucleus, is not down-regulated in our \textit{Irs2}^{-/-} murine \(\beta\)-cells with a C57BL/6J background. In addition, surprisingly, even in \textit{Irs2}^{-/-} mice on a high fat diet with markedly elevated blood glucose, exceeding 400 mg/dl, Pdx-1 expression was not reduced. Furthermore, we found Pdx-1 to be markedly decreased in severely diabetic \textit{Irs2}^{-/-} mice with a mixed C57BL/6J \(\times\) 129Sv background. We conclude that Pdx-1 expression in \textit{Irs2}^{-/-} mice is regulated in a strain-dependent manner, and our results suggest that a certain 129Sv-related genetic component(s) is involved in reduced Pdx-1 expression, leading to severe diabetes in \textit{Irs2}^{-/-} mice.

**EXPERIMENTAL PROCEDURES**

**Animals**—\textit{IRS}-2-deficient mice were generated as previously described (11). \textit{Irs2}^{-/-} mice and \textit{Irs2}^{-/-} mice had a C57BL/6J background, except when specifically stated to have a mixed C57BL/6J \(\times\) 129Sv background. Mice were housed under a 12-h light/dark cycle and given \textit{ad libitum} access to food. All experiments in this study were performed on male mice.

**Isolation of Islets**—Isolation of islets from \textit{Irs2}^{-/-} mice and \textit{Irs2}^{-/-} mice was carried out as described elsewhere (38). In brief, after clamp ing the common bile duct at a point close to the duodenal outlet, 2.5 ml of Krebs-Ringer bicarbonate buffer (129 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\), 5 mM NaHCO\(_3\), and 10 mM HEPES at pH 7.4) containing 4 mg/ml collagenase (Sigma) was injected into the duct. The swollen pancreas was removed and incubated at 37 °C for 3.5 min. The pancreas was dispersed by pipetting and washed twice with ice-cold Krebs-Ringer bicarbonate buffer. RNase inhibitor (Invitrogen) was added in buffer if RNA extraction was required. Islets were manually collected through a stereoscopic microscope.

**RNA Preparation and Real Time Quantitative PCR**—Total RNA was extracted from isolated islets with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. After treatment with DNase I (Invitrogen) for the removal of genomic DNA, cDNA synthesis was performed using the SuperScript Preamplification System (Invitrogen) and then TaqMan (Applied Biosystems, Foster City, CA) quantitative PCR (50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min) was performed with the ABI Prism 7000 PCR instrument (Applied Biosystems) to amplify samples for the Pdx1, Hnf5, Hnf4, cytochrome, \(\beta\)-actin, GAPDH, Glut2, and \textit{Nkx6.1} genes. Primers and probes were as follows: Pdx1 forward, CCGAGACATCATAAAACTGG; Pdx1 reverse, CCAGCTGACTCATGTCCATTCA; Pdx1 probe, CTGGTGAAGGGCCAGGACA; Hnf4a forward, CACCAAGCCCCTGCTCAACACTG; Hnf4a reverse, GATGAGCAGGCAAGTGCAGATAAAA; cytochrome forward, GGTCCTGGCATCTTGAGCA; cytochrome reverse, GTACGTCGAGCTCTCTTGT; GAPDH forward, ACCGTCTCTCGAACCGG; GAPDH reverse, ATCGTCCCTGCGACCGACAG; \textit{Nkx6.1} forward, ATCGTCCCTGCGAGCCTCAGAG; \textit{Nkx6.1} reverse, ATCGTCCCTGCGAGCCTCAGAG; \textit{Nkx6.1} probe, AGGTAGCACTTCAACACCCCAGC; \textit{Nkx6.1} beta-actin forward, GGCACAACCGTTGAAGTGA; \textit{Nkx6.1} beta-actin reverse, CAGACCGCTGGATGTTGACACGT; \textit{Nkx6.1} beta-actin probe, TTTGAGACCTTCAACACCCCAGC; Glut2 forward, TGGTGAAGGGCCAGGACA; Glut2 reverse, GATGAGCAGGCAAGTGCAGATAAAA; \textit{Nkx6.1} forward, TGGTGAAGGGCCAGGACA; \textit{Nkx6.1} reverse, ACAAACCTCTCAGA; \textit{Nkx6.1} probe, CCAGGACCGGAGTCTCCTTAGTAC; \textit{Nkx6.1} beta-actin forward, GATGAGCAGGCAAGTGCAGATAAAA; \textit{Nkx6.1} beta-actin reverse, CAGACCGCTGGATGTTGACACGT; \textit{Nkx6.1} beta-actin probe, TTTGAGACCTTCAACACCCCAGC.

**Immunohistochemistry**—The removed pancreata were immediately immersion-fixed in Bouin’s solution at 4 °C overnight. Tissues were routinely processed for paraffin embedding, and 2-μm sections were cut and mounted on silanized slides. With the indicated antibodies, the sections were immunologically stained brown using an Envision labeling Polymer Kit (DakoCytomation Co. Ltd., Kyoto, Japan) with a DAB Substrate Kit (3.3’-diaminobenzidine) (Vector Laboratories, Burlingame, CA) and/or stained red using an alkaline phosphatase-conjugated goat anti-rabbit IgG antibody with Vector Red (Vector Laboratories). Western Blot Analysis—Isolated islets were homogenized in ice-cold buffer A (25 mM Tris-HCl (pH 7.4), 10 mM sodium orthovandenate, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride). The protein concentration of lysates was equaled for loading by total protein assay (Pierce). SDS samples were separated on polyacrylamide gels and transferred to a Protran nitrocellulose membrane (Schleicher & Schuell). The membrane was incubated with the indicated antisera and horseradish peroxidase-conjugated anti-IgG antibodies. Bands were detected by ECL detection reagents (Amersham Biosciences). Antibodies—Rabbit anti-N-terminal Pdx-1 antiserum (21) and rabbit anti-Nkx6.1 antiserum (21) were produced as previously described. Guinea pig anti-insulin, anti-insulin polypeptide, and anti-rabbit somatostatin, and rabbit anti-peroxidase polypeptide antibodies were purchased from DakoCytomation Co. Ltd. Rabbit anti-Glut2 antibody was purchased from Chemicon International, Inc. (Temecula, CA). Goat anti-HNF3β and goat anti-actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**High Fat Diet Experiment**—Six-week-old male \textit{Irs2}^{-/-} mice and \textit{Irs2}^{-/-} mice were housed in individual cages. They were divided into two groups, a regular chow group and a high fat diet group. The high fat diet was prepared with the following composition: 32% safflower oil, 33.1% casein, 17.6% sucrose, 1.4% vitamins, 9.8% minerals, 5.6% cellulose, 0.5% niacin, as previously described (40). Materials were purchased from Oriental Yeast Co., Ltd. (Osaka, Japan). Body weight was assessed between 9:00 and 11:00. After 5 weeks of feeding, blood samples were taken from the orbital sinus, and glucose was measured using an automatic blood glucose meter (Glutest Pro; Sanwa Chemical, Nagoya, Japan) between 9:00 and 11:00 in the fed state and after fasting for 16 h from 19:00 to obtain fasted state values. Whole blood was collected and centrifuged in heparinized tubes, and the plasma was stored at −20 °C. Insulin levels were determined using an insulin radioimmunoassay kit (BIOTRACK, Amersham Biosciences) with rat insulin as a standard (40).

Values are presented as means ± S.E. The statistical significance of differences between groups was determined by Student’s \(t\) test (two-tailed).
RESULTS

Expression of Pdx-1 in Irs2<sup>−/−</sup> Murine β-Cells—Pdx-1 mRNA levels were measured in islets from Irs2<sup>−/−</sup> mice by real-time quantitative PCR. This study did not yield conclusive results, because Pdx-1 mRNA levels in Irs2<sup>−/−</sup> murine islets were unchanged when β-actin and GAPDH were used as an internal control.
internal standard and were increased when cyclophilin was used (Fig. 1a).

Western blot analysis also showed that Pdx-1 protein was not decreased in isolated islets from Irs2火锅 mice (Fig. 1b). In addition, immunohistochemical studies revealed Pdx-1 protein to be equally expressed in the nuclei of Irs2火锅 murine β-cells at 8 weeks of age, as compared with those of Irs2火锅 mice (Fig. 2). These results suggested the IRS-2 signal not to always be required for normal Pdx-1 expression.

Expression of Pdx-1-regulating Transcription Factor Genes in Irs2火锅 Murine β-Cells—HNF3β and HNF1α reportedly regulate the expression of Pdx-1 in vivo and in vitro (33). Contrary to a previous report (37), real time quantitative PCR study revealed Hnf3β and Hnf1α mRNA expressions to not be decreased when β-actin was selected as an internal control (Fig. 3a). HNF3β protein expression was not reduced in islets from 8-week-old Irs2火锅 mice (Fig. 3b).

Expression of Pdx-1-regulated Downstream Genes in Irs2火锅 Murine β-Cells—Pdx-1 regulates the expressions of various pancreatic genes, including insulin, glucose transporter type 2 (Glut2) (29, 41, 42), and Nkx6.1 (32, 33). The immunohistochemical results with anti-insulin, anti-Glut2, and anti-Nkx6.1 antibodies showed expression of these three proteins to be well preserved in Irs2火锅 murine islets, consistent with our previous observation that both glucose-induced insulin secretion and insulin contents were preserved (11) (Figs. 2, a and b, and 4, a–d). Real time quantitative PCR study also indicated that Glut2 and Nkx6.1 mRNA expressions to not be decreased (Fig. 4e).

The Effect of Hyperglycemia Induced by Aging or a High Fat Diet on Pdx-1 Expression in Irs2火锅 Murine β-Cells—Irs2火锅 mice progressively develop hyperglycemia starting at 10 weeks (11). To clarify the effect of aging or glucose toxicity on Pdx-1 expression, immunohistochemistry was performed on pancreata of 30-week-old diabetic Irs2火锅 mice. Pdx-1 protein was not reduced in the nuclei of β-cells from old Irs2火锅 mice (Fig. 5). Irs2火锅 mice on a high fat diet showed marked aggravation of glucose intolerance with exacerbation of obesity (Table 1). Even in this state, the expression of Pdx-1 protein in β-cells, as well as those of downstream genes such as insulin and Glut2, did not show apparent reduction (Fig. 6, A and B).

Severe Diabetes Seen in Irs2火锅 Mice with a C57BL/6J × 129Sv Mixed Background—We have maintained Irs2火锅 mice with a C57BL/6J or a C57BL/6J × CBA hybrid background. We intercrossed our C57BL/6J background Irs2火锅 mice with mice having a C57BL/6J × 129Sv hybrid background. Irs2火锅 mice with a C57BL/6J × 129Sv hybrid background were divided in two groups based on blood glucose level; some Irs2火锅 mice showed mild hyperglycemia, as did those with only a C57BL/6J background, whereas other Irs2火锅 mice had very severe diabetes, dying due to dehydration with polyuria. Immunohistochemical studies revealed Pdx-1 protein to be generally undetectable as well as insulin in β-cells from these mice, whereas it was well preserved in mildly diabetic Irs2火锅 mice (Fig. 7).

DISCUSSION

The results of this study show Pdx-1 expression not to be down-regulated in β-cells from our Irs2火锅 mice with a C57BL/6J background through real time PCR, immunohistochemistry, and Western blot analysis, consistent with our previous report (11) showing increased glucose-induced insulin secretion and comparable insulin content in our Irs2火锅 murine islets. In addition, expression of genes lying upstream from Pdx-1, such as HNF3β and HNF1α, as well as that of downstream target genes, including insulin, Glut2, and Nkx6.1, was well preserved in our Irs2火锅 murine β-cells.

Then what factors are involved in severely reduced Pdx-1 expression in β-cells from Irs2火锅 mice, as previously reported by another laboratory (37)? The involvement of glucose toxicity has been suggested. Their Irs2火锅 mice have been reported to show a wide range of fasting blood glucose levels (lower group, 99 ± 4 mg/dl; higher group, 290 ± 22 mg/dl) at 8–10 weeks of
age (9, 43), whereas our Irs2−/− mice show comparatively lower (∼100 mg/dl) levels at 10 weeks of age (11). Hyperglycemia can down-regulate Pdx-1 expression (44, 45), thereby impairing β-cell function and further aggravating hyperglycemia. However, our study revealed that, in 30-week-old diabetic Irs2−/− mice, there was no decrease in Pdx-1 expression in β-cells. In

Table I

Characterization of Irs2−/− mice on a regular chow diet or a high fat diet

The high fat diet experiment was performed as described under “Experimental Procedures.” Six-week-old male Irs2+/+ mice and Irs2−/− mice were given ad libitum access to regular chow or a high fat diet for 5 weeks. Values are presented as means ± S.E. (n = 6 per group). BW, body weight; ΔBW, change in BW during the 5-week period; BG, blood glucose. *, p < 0.05 versus regular chow group with the same genotype.

| Component          | Irs2+/+ Chow | Irs2+/+ High Fat | Irs2−/− Chow | Irs2−/− High Fat |
|--------------------|--------------|------------------|--------------|------------------|
| BW (g)             | 26.4 ± 0.3   | 27.5 ± 0.8       | 27.3 ± 0.5   | 31.3 ± 1.1*     |
| ΔBW (g)            | 7.2 ± 0.4    | 8.3 ± 0.8        | 9.9 ± 0.4    | 13.9 ± 0.7*     |
| BG (fed) (mg/dl)   | 147 ± 4      | 249 ± 8*         | 223 ± 12     | 429 ± 17*       |
| BG (fasted) (mg/dl)| 74 ± 5       | 98 ± 7*          | 106 ± 9      | 203 ± 5*        |
| Insulin (fasted) (ng/ml) | 0.42 ± 0.08 | 0.48 ± 0.12     | 0.97 ± 0.10  | 2.12 ± 0.23*    |

Fig. 6. Pdx-1 expression is not down-regulated in islets from Irs2−/− mice on a high fat diet for 5 weeks. A, representative sections of pancreata from Irs2+/+ and Irs2−/− mice on a normal chow diet or a high fat diet, immunostained with antibodies against insulin, glucagon, somatostatin, pancreatic polypeptide, Pdx-1, and Glut2. Pancreata from four or five mice per group were examined. a–d, insulin is represented by brown staining, and non-β-cells are represented by red staining with antibodies against glucagon, somatostatin, and pancreatic polypeptide, e–h, Pdx-1 is represented by brown staining, i–l, Glut2 is represented by red staining. B, Western blots of Pdx-1 in islets isolated from Irs2+/+ and Irs2−/− male mice on normal chow diet or a high fat diet. For each group, islets from two mice were pooled, and lysates (15 μg of protein) were subjected to Western blot analysis with antibodies against Pdx-1 and actin as an internal control.
addition, even in Irs2<sup>−/−</sup> mice on a high fat diet with blood glucose levels over 400 mg/dl, Pdx-1 expression was not down-regulated. Thus, hyperglycemia itself may not be a major factor down-regulating Pdx-1 expression in β-cells from our Irs2<sup>−/−</sup> mice.

Another difference between their Irs2<sup>−/−</sup> mice and ours is the genetic background (C57BL/6J × 129Sv versus C57BL/6J). Some previous reports have demonstrated that genetic backgrounds can affect the severity of phenotype in knockout mouse models (46–48). We suspected that certain genetic 129Sv components might be involved in the Pdx-1 expression in Irs2<sup>−/−</sup> mice, either directly or indirectly as modifier genes. When our Irs2<sup>−/−</sup> mice were crossed with mice having a 129Sv background, a significant proportion of F<sub>2</sub> mice developed severe diabetes and died before several months of age, although others remained mildly diabetic and lived at least 1 year. When mildly diabetic F<sub>2</sub> mice were crossed with 129Sv, the proportion of severely diabetic Irs2<sup>−/−</sup> mice was larger. Thus, certain genetic components in 129Sv mice apparently contribute to the development of severe diabetes (49). We found Pdx-1 expression to be reduced in β-cells from severely diabetic Irs2<sup>−/−</sup> mice (Fig. 7). Our data strongly suggest that IRS-2 is not always required for the normal expression and function of Pdx-1, because disruption of IRS-2 did not affect the expression of Pdx-1 and its downstream target genes in our knockout mouse β-cells with a C57BL/6J background even when severe hyperglycemia was induced by a high fat diet. Cross-breeding experiments revealed that Pdx-1 reduction in Irs2<sup>−/−</sup> mouse β-cells is a strain-dependent phenomenon. Kushner et al. (37) demonstrated that Pdx-1 expression is already reduced in their Irs2<sup>−/−</sup> islets at birth, suggesting that Pdx-1 expression in β-cells is regulated via IRS-2 signaling in their mouse models. Considering their results and ours, when the genetic 129Sv components are present, IRS-2 might regulate the expression of Pdx-1. Another hypothesis is that certain 129Sv-related genetic elements may be involved in Pdx-1 reduction by enhancing the inhibitory effect of chronic hyperglycemia on the expression of Pdx-1, since Pdx-1 is a potential target of the glucose toxicity (44, 50). This hypothesis might explain the mechanisms of age-related intense aggravation on β-cell dysfunction in their Irs2<sup>−/−</sup> mice (9, 10, 37). Further study is anticipated to clarify this issue.

Then what common pathways are responsible for β-cell growth failure in their and our Irs2<sup>−/−</sup> mice? β-cell-specific constitutively active Akt-1 transgenic mice reportedly have an increased β-cell mass (19, 20) and suggest the involvement of IRS-2/Akt pathway in β-cell mass regulation (51). The Foxo1 pathway might also be involved in β-cell growth failure in Irs2<sup>−/−</sup> mice. In addition to its role in metabolic regulation, Foxo1 might be involved in β-cell growth regulation as a downstream effector of IRS-2/Akt (52).

Our observations suggest that Pdx-1 reduction in Irs2<sup>−/−</sup>-mature β-cells is a strain-dependent event, and, at least with a C57BL/6J background, Irs2<sup>−/−</sup> is not a major regulator of Pdx-1 expression in mouse β-cells. The mechanisms of β-cell growth failure, which is commonly observed in Irs2<sup>−/−</sup> mice with any background, should be distinguished from insulin secretory dysfunction with Pdx-1 reduction.

Acknowledgments—We thank Dr. Shinya Nagamatsu, Dr. Wataru Ogawa, Dr. Mitsuhiro Noda, Dr. Kazuo Hara, Masashi Aoyama, Dr. Atsushi Inoue, Dr. Kentaro Sakamoto, and Dr. Katsuko Takasawa for helpful discussion. We thank N. Kowatari-Otsuka, A. Nagano, and H. Chiyonobu for excellent technical assistance and mouse husbandry.

REFERENCES
1. Kadotani, T. (2000) J. Clin. Invest. 106, 459–465
2. White, M. F. (1998) Mol. Cell. Biochem. 182, 3–11
3. Virkamaki, A., Ueki, K., and Kahn, C. R. (1999) J. Clin. Invest. 103, 931–943
4. Saltiel, A. R., and Kahn, C. R. (2001) Nature 414, 799–806
5. Nakae, K., Kido, Y., and Accili, D. (2001) Endo Rev. 22, 813–835
6. Shulman, G. I. (2000) J. Clin. Invest. 106, 171–176
7. Tamemoto, H., Kadotani, T., Tobe, K., Tagi, T., Sakura, H., Hayasaka, T., Terasuchi, Y., Ueki, K., Kaburagi, Y., Satoh, S., Sekihara, H., Yoshinaka, S., Horikoshi, H., Furuta, Y., Iwata, Y., Kasuga, M., Yasaki, Y., and Aizawa, S. (1994) Nature 372, 182–186
8. Araki, E., Iwata, K., Akita, M., Muranaka, S., Hara, K., Shulman, G. I., Bonner-Weir, S., and White, M. F. (1998) Nature 391, 900–904
9. Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J., Ren, J. M., Previs, S., Zhang, Y., Bernal, D., Pena, S., Shulman, G. I., Bonner-Weir, S., and White, M. F. (1999) Nature 391, 900–904
10. Withers, D. J., Burks, D. J., Towery, H. H., Altamura, S. L., Flint, C. L., and White, M. F. (1999) Nat. Genet. 23, 32–40
11. Kudoh, N., Tobe, K., Terasuchi, Y., Eto, K., Yamauchi, T., Satake, R., Tsubamoto, Y., Ueki, K., Nakano, R., Miki, H., Satoh, S., Sekihara, H., Schlichting, S., Leniak, M., Aizawa, S., Nagai, R., Kimura, S., Akamizu, Y., Taylor, J. L., and Kadotani, T. (2000) Diabetes 49, 1888–1899
12. Kido, Y., Burks, D. J., Withers, D., Bruning, J. C., Kahn, C. R., White, M. F., and Accili, D. (2000) J. Clin. Invest. 106, 199–205
Preserved Pdx1 Expression in Irs2<sup>−/−</sup> β-cells with B6 Background

13. Accili, D. (2001) J. Clin. Invest. 108, 1557–1576
14. Saltiel, A. R. (2001) Cell 104, 517–529
15. Kulkarni, R. N., Bruning, J. C., Winnay, J. N., Postic, C., Magnuson, M. A., and Kahn, C. R. (1999) Cell 96, 329–339
16. Kulkarni, R. N., Holzenberger, M., Shih, D. Q., Ozcan, U., Stoffel, M., Magnuson, M. A., and Kahn, C. R. (2002) Nat. Genet. 31, 111–115
17. Xuan, S., Kitamura, T., Nakae, J., Politi, K., Kuo, Y., Fisher, P. E., Murroni, M., Cinti, S., White, M. F., Herrara, P. L., Accili, D., and Efstratiadis, A. (2002) J. Clin. Invest. 110, 1011–1019
18. Pende, M., Kozma, S. C., Jaquet, M., Oorschot, V., Burocilin, R., Marchand-Brustel, Y. L., Klumperman, J., Thorens, B., and Thomas, G. (2000) Nature 408, 994–997
19. Tuttle, R. L., Gill, N. S., Pugh, W., Lee, J. P., Koeberlein, B., Forth, E. E., Polonsky, K. S., Naji, A., and Birnbaum, M. J. (2001) Nat. Med. 7, 1133–1137
20. Bernal-Mizrachi, E., Wen, W., Stahlhut, S., Welling, C., and Permuth, M. (2001) J. Clin. Invest. 108, 1631–1638
21. Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L. M., and Wright, C. V. E. (1996) Development 122, 983–995
22. Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) Nature 371, 606–609
23. Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., and Edlund, H. (1998) Genes Dev. 12, 1763–1768
24. Edlund, H. (1998) Diabetes 47, 1817–1823
25. Brisseva, M., Shiota, M., Nicholson, W. E., Gannon, M., Knob, S. M., Piston, D. W., Wright, C. V. E., and Powers, A. C. (2002) J. Biol. Chem. 277, 11225–11232
26. Dutta, S., Bonner-Weir, S., Montminy, M., and Wright, C. (1998) Nature 392, 560
27. Duncan, S. A., Navas, M. A., Dufort, D., Rossant, J., and Stoffel, M. (1998) Science 281, 692–695
28. Peers, B., Leonard, J., Sharma, S., Teitelman, G., and Montminy, M. R. (1994) Mol. Endocrinol. 10, 1327–1334
29. Waeber, G., Thompson, N., Nicod, P., and Bonny, C. (1996) Mol. Endocrinol. 10, 1798–1806
30. Watada, H., Kajimoto, Y., Miyagawa, J., Hanafusa, T., Hamaguchi, K., Matsusaka, T., Yamamoto K., Matsuwasa, Y., Kawamori, R., and Yamasaki, Y. (1996) Diabetes 45, 1826–1831
31. Watada, H., Kajimoto, Y., Omuyahara, Y., Matsusaka, T., Kuretto, H., Fujitani, Y., Kamada, T., Kawamori, R., and Yamasaki, Y. (1996) Diabetes 45, 1478–1488
32. Watada, H., Mirmira, R. G., Leung, J., and German, M. S. (2000) J. Biol. Chem. 275, 34224–34230
33. Shih, D. Q., Heimesaat, M., Kuwajima, S., Stein, R., Wright, C. V. E., and Stoffel, M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 3818–3823
34. Shih, D. Q., and Stoffel, M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 14189–14191
35. Stoffels, D. A., Ferrer, J., Clarke, W. L., and Habener, J. F. (1997) Nat. Genet. 17, 158–139
36. Stoffels, D. A., Staasejovic, V., and Habener, J. F. (1998) J. Clin. Invest. 102, 232–241
37. Kushner, J. A., Ye, J., Schubert, M., Burks, D. J., Dow, M. A., Flint, C. L., Dutta, S., Wright, C. V. E., Montminy, M. R., and White, M. F. (2002) J. Clin. Invest. 109, 1193–1201
38. Eto, K., Tsuhamoto, Y., Terauchi, Y., Sugiyama, T., Kishimoto, T., Takahashi, N., Yamauchi, N., Kubota, N., Murayama, S., Aizawa, T., Akanuma, Y., Aizawa, S., Kasai, H., Yazaki, Y., and Kadowaki, T. (1999) Science 283, 981–985
39. Susel, L., Kalamars, J., Hartigan-O’Corner, D. J., Meneses, J. J., Pedersen, R. A., Rubenstein, J. L., and German, M. S. (1998) Development 125, 2213–2221
40. Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Sato, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsuhamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Fujita, T., Ezaki, O., Aizawa, S., Nagai, R., Tohe, K., Kimura, S., and Kadowaki, T. (1999) Mol. Cell 4, 597–609
41. Bonny, C., Roduit, B., Gremlich, S., Nicod, P., Thorens, B., and Waeber, G. (1997) Mol. Cell. Endocrinol. 135, 59–65
42. Wang, H., Meechler, P., Ritz-Laser, B., Hagenfeldt, K. A., Ishihara, H., Philippe, J., and Wollheim, C. B. (2001) J. Biol. Chem. 276, 25279–25286
43. Higaki, Y., Wojtaszewski, J. F. P., Hirschman, M. F., Withers, D. J., Towner, H. L., White, M. F., and Goodyear, L. J. (1999) J. Biol. Chem. 274, 20791–20795
44. Poutoat, V., and Robertson, R. P. (2002) Endocrinology 143, 339–342
45. Harmon, J. S., Gleeson, C. E., Tanaka, Y., Oseid, E. A., Hunter-Berger, K. K., and Robertson, R. P. (1999) Diabetes 48, 1995–2000
46. Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J., and Efstratiadis, A. (1993) Cell 75, 59–72
47. Qiu, J., Ogas, S., Mounzib, K., Ewart-Toland, A., and Chehab, F. F. (2001) Endocrinology 142, 3421–3425
48. Huang, H. P., Chu, K., Nemoz-Guillard, E., Elberg, D., and Tsai, M. J. (2002) Mol. Endocrinol. 16, 541–551
49. Terauchi, Y., Matsui, J., Suzuki, R., Kubota, N., Komeda, K., Aizawa, S., Eto, K., Kimura, S., Nagai, R., Tohe, K., Lienhard, G. E., and Kadowaki, T. (2003) J. Biol. Chem. 278, 14284–14290
50. Jonas, J. C., Sharma, A., Hasenkamp, W., Ilkova, H., Putane, G., Laybutt, R., Bonner-Weir, S., and Weir, G. C. (1999) J. Biol. Chem. 274, 14112–14121
51. Jetton, T. L., Liu, Y. Q., Trotman, W. E., Nevin, P. W., Sun, X.-J., and Leedh, J. L. (2001) Diabetologia 44, 2066–2065
52. Nakae, J., Biggs, W. H., Kitamura, T., Cavenee, W. K., Wright, C. V. E., Arden, K. C., and Accili, D. (2002) Nat. Genet. 32, 245–253