Pancreatic β Cell Mass Preserved in Heterozygous PDK1 Knockout Mice

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We have demonstrated that 3-phosphoinositide-dependent protein kinase 1 (PDK1) contributes to signaling by insulin or insulin-like growth fctor-1 (IGF-1) that is responsible for the regulation of both the number and size of pancreatic β cells in mice. Complete ablation of PDK1 in pancreatic β cells leads to progressive hyperglycemia as a result of loss of β cell mass. In this study, we generated heterozygous pancreatic β cell-specific PDK1 knockout (βPDK1$^{+/-}$) mice and fed them a high-fat diet as a model of human type 2 diabetes. The βPDK1$^{+/-}$ mice exhibited normal glucose tolerance even on a high-fat diet. Further, islet morphology and β cell mass were normal in βPDK1$^{+/-}$ mice, and haploinsufficiency of PDK1 did not impair the compensatory hyperplasia of β cells on a high-fat diet. The phosphorylation and expression of the molecules that are expressed downstream of PDK1 were similar in the islets of the βPDK1$^{+/-}$ and control mice. Eventually, we concluded that glucose homeostasis and islet mass were maintained in βPDK1$^{+/-}$ mice.

The mass of pancreatic islets is decreased in individuals with type 2 diabetes (2), suggesting that it is the cause of the disease. Signaling pathways triggered by insulin or insulin-like growth factor-1 (IGF-1) have been implicated in the maintenance of islet mass (5,9,10,15,16). We have demonstrated that 3-phosphoinositide-dependent protein kinase 1 (PDK1) contributes to signaling by insulin or IGF-1 that is indispensable for the survival and maintenance of pancreatic β cells in mice (5). However, considering that type 2 diabetes patients might have mutations in a single allele, it is important to examine the effect of haploinsufficiency of PDK1 on the function and/or mass of pancreatic β cells. Therefore, we generated mice in which the *pdk1* gene is deleted by ~50%, specifically in pancreatic β cells (βPDK1$^{+/-}$ mice). This is described in Materials and Methods. Furthermore, we examined the ability of β cells to compensate for the insulin resistance in βPDK1$^{+/-}$ mice.

MATERIALS AND METHODS

Mice

We generated heterozygous pancreatic β cell-specific PDK1 knockout (βPDK1$^{+/-}$) mice by crossing *Pdk1$^{flox/flox}$* mice (7) with those that express the *Cre* recombinase gene under the

control of rat insulin-2 gene (6). The animals were maintained and blood glucose and plasma insulin concentrations were determined as described previously (4,8).

Only male mice were used for the experiments. This study was performed according to the guidelines of the Animal Ethics Committee of Kobe University Graduate School of Medicine.

Oral glucose tolerance test

The mice were deprived of food for 16 h. Blood was collected immediately before and 15, 30, 60, and 120 min after the oral administration of glucose (1.5 mg/g).

Immunoblot analysis

Lysates of isolated islets that had been either stimulated (or not) with 100 nM recombinant human IGF-1 (Pepro Tech) were prepared as previously described (5,14). The lysates were probed with antibodies against PDK1, Akt, the phosphor-Thr^{308} and phospho-Ser^{473} forms of Akt, Foxo1, the phosphor-Thr^{24} form of Foxo1, S6, the phosphor-Ser^{235} and phosphor-Ser^{236} forms of S6, 4E-BP1, the phosphor-Thr^{37} and phosphor-Thr^{46} forms of 4E-BP1, and the phosphor-Thr^{202} and phosphor-Thr^{204} forms of p42/44 mitogen-activated protein kinase (MAPK) (all from Cell Signaling). Antibodies against β-actin (Sigma-Aldrich) were also used.

Immunostaining and morphometric analysis of islets

We subjected 3–5 mice of each genotype at the indicated age to morphometric analysis. Their pancreatic sections were subjected to 2-color immunofluorescence staining with antibodies against insulin and glucagon (both from Dako). For the morphometric analysis, the islets were manually traced and analyzed on images obtained using WinROOF software (Mitani). The cross-sectional area of the islets with more than 5 insulin-positive cells was measured in at least 3 sections separated by 200 μm. The total β cell mass was calculated as the total area of β cells expressed as a percentage of the total area of the pancreas.

Statistical analysis

Data are presented as means ± standard error of the mean (SEM) and compared by analysis of variance.

RESULTS

Similar to our previous report (5), the βPDK1$^{-/-}$ mice used in this study exhibited severe hyperglycemia and hypoinsulinemia. However, there were no significant differences in the fed blood glucose and plasma insulin concentrations between the βPDK1$^{+/-}$ and control mice until 24 weeks of age (Fig. 1A). Next, we performed the oral glucose tolerance test in 24-week-old animals that had been deprived of food overnight. The βPDK1$^{+/-}$ mice also exhibited normal glucose tolerance; the levels of blood glucose and plasma insulin after glucose load did not alter significantly in these mice as compared with in the control animals (Fig. 1B).

To investigate the effect of PDK1 haploinsufficiency on β cell function in the insulin-resistant state, the control and βPDK1$^{+/-}$ mice were fed a high-fat diet for 21 weeks from weaning. The increases in body weight were similar for the 2 types of mice on this diet (data not shown). Random measurement of blood glucose and plasma insulin concentrations in the fed state also did not reveal any significant differences between the βPDK1$^{+/-}$ and control mice (Fig. 1C). Oral glucose tolerance tests were performed to detect any subtle abnormality in glucose homeostasis that would not result in overt hyperglycemia. However, the blood glucose and plasma insulin levels after oral glucose load were observed to be similar in the control and βPDK1$^{+/-}$ mice (Fig. 1D). These results indicate that

haploinsufficiency of PDK1 in pancreatic β cells does not apparently affect glucose homeostasis.
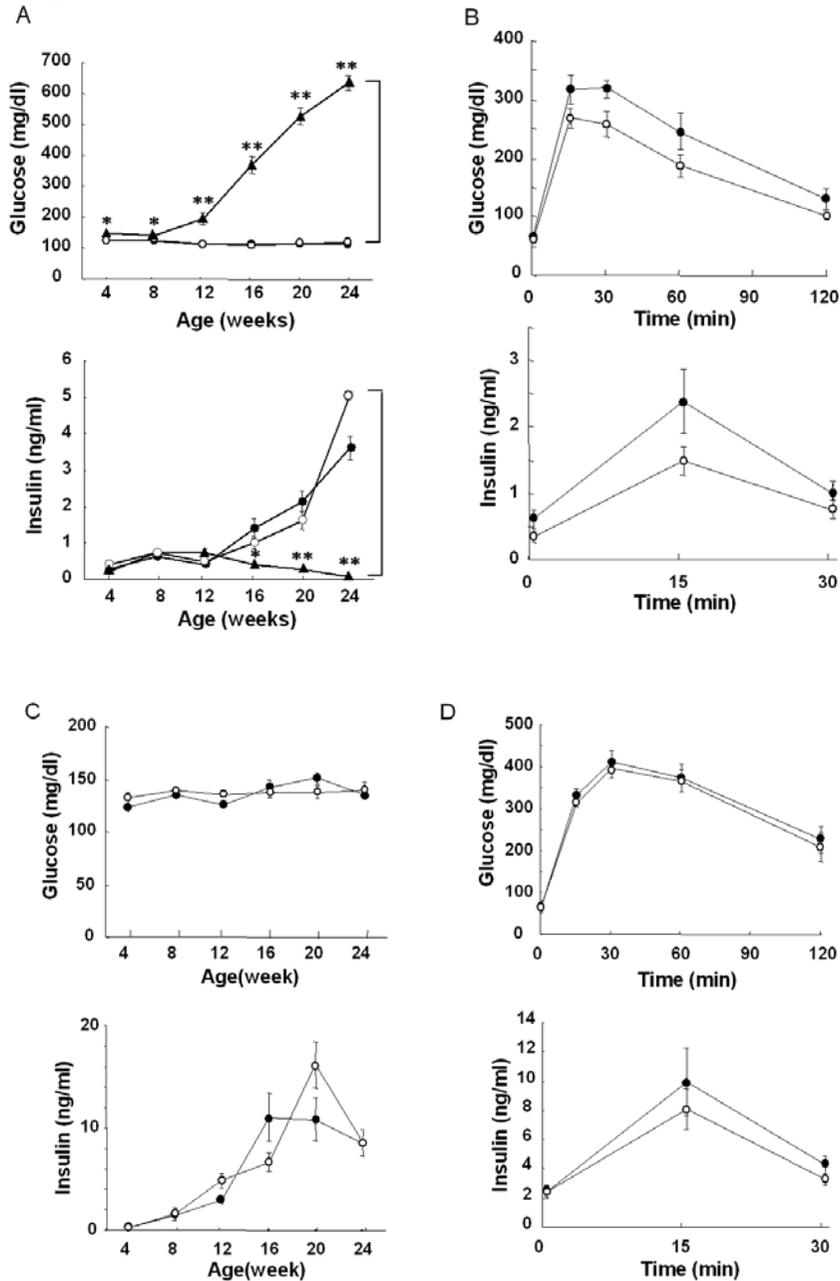
Figure 1 Effect of β cell-specific haploinsufficiency of PDK1 on glucose metabolism. (A) Blood glucose and plasma insulin concentrations of control (open circles), βPDK1$^{-/-}$ (solid triangles), and βPDK1$^{+/-}$ (solid circles) mice in the fed state. (B) Results of oral glucose tolerance tests of 24-week-old control (n=9) and βPDK1$^{+/-}$ (n=11) mice that had fasted overnight. (C) Blood glucose and plasma insulin concentrations of control and βPDK1$^{+/-}$ mice that were on a high-fat diet. (D) Results of oral glucose tolerance tests of control (n=10) and βPDK1$^{+/-}$ (n=16) mice fed a high-fat diet for 20 weeks. Data represented as means ± SEM.

Next, we determined the possible effects of β cell-specific haploinsufficiency of PDK1 on pancreatic morphology. Immunostaining of pancreatic sections from 24-week-old animals with antibodies against insulin and glucagon revealed normal islet architecture in the βPDK1$^{+/-}$ mice (Fig. 2A). Quantitative analysis also revealed no significant difference in the pancreatic β cell area between the control and βPDK1$^{+/-}$ mice (Fig. 2B). The size of individual β cells was also similar in the control and βPDK1$^{+/-}$ mice (Fig. 2C). We also examined islet morphology in mice that were fed a high-fat diet. Islet mass in the βPDK1$^{+/-}$ mice fed the high-fat diet was similar to that in the control animals on this diet (Fig. 2D). These results indicate that the β cells of βPDK1$^{+/-}$ mice can grow like those of the control mice during development of insulin resistance.

Immunoblot analysis revealed that the amount of PDK1 was ~50% lower in the islets of βPDK1$^{+/-}$ mice than in those of the control mice (Fig. 3). Next, we examined the expression and phosphorylation of molecules that are believed to function downstream of PDK1 (Fig. 3). PDK1 phosphorylates Akt on Thr^{308}, and the immunoblot analysis showed that the amount of Akt with phosphorylated Thr^{308} did not differ between the islets of βPDK1$^{+/-}$ mice and those of the control animals. Although PDK1 does not target Ser^{473} of Akt, the amount of Akt phosphorylated on this residue was also found to be similar in the control and βPDK1$^{+/-}$ mice. Therefore, the activity of Akt in the islets of βPDK1$^{+/-}$ mice appeared to be comparable to that in the islets of control mice. Akt phosphorylates Foxo1, and the abundance of Foxo1 with phosphorylated Thr^{24} was similar in the 2 genotypes. The activity of mammalian target of rapamycin complex 1 (mTORC1) indicated by the phosphorylation of p70 S6 kinase on Thr^{389}, S6 on Ser^{235} and Ser^{236}, and 4E-BP1 on Thr$^{37/46}$ also did not differ between the control and βPDK1$^{+/-}$ mice. Furthermore, haploinsufficiency of PDK1 did not affect the phosphorylation of mitogen-activated protein (MAP) kinase downstream of Ras. These results indicate that haploinsufficiency of PDK1 does not impair the activity of proteins downstream of PDK1, resulting in the maintenance of β cell mass and glucose homeostasis.

DISCUSSION

Although the regulation of islet mass is complex, recent studies have suggested the importance of a signaling pathway that includes the insulin or IGF-1 receptors, insulin receptor substrate, and phosphatidylinositol (PI) 3-kinase (9,10,16). PDK1 is a serine-threonine kinase that mediates signaling downstream of PI 3-kinase; it then phosphorylates and activates several members of the cAMP-dependent, cGMP-dependent, protein kinase C (AGC) family of kinases, including Akt and p70 S6 kinase (1). PDK1 is considered to be encoded by a single gene, and no isoform of this protein has been detected to date. Other researchers have demonstrated that PDK1-deficient mice systemically die *in utero* at embryonic day 9.5 (11). They have also established hypomorphic PDK1 mice that express ~10% of the normal level of PDK1. Hypomorphic PDK1 mice are viable and fertile and show normal insulin-induced activity of Akt and p70 S6 kinase. However, interestingly, these mice are 40–50% smaller than control mice, mainly due to reduced cell size and not reduced cell number. Therefore, PDK1 might regulates cell size rather than cell number and the insulin-induced activation of Akt and p70 S6 kinase.
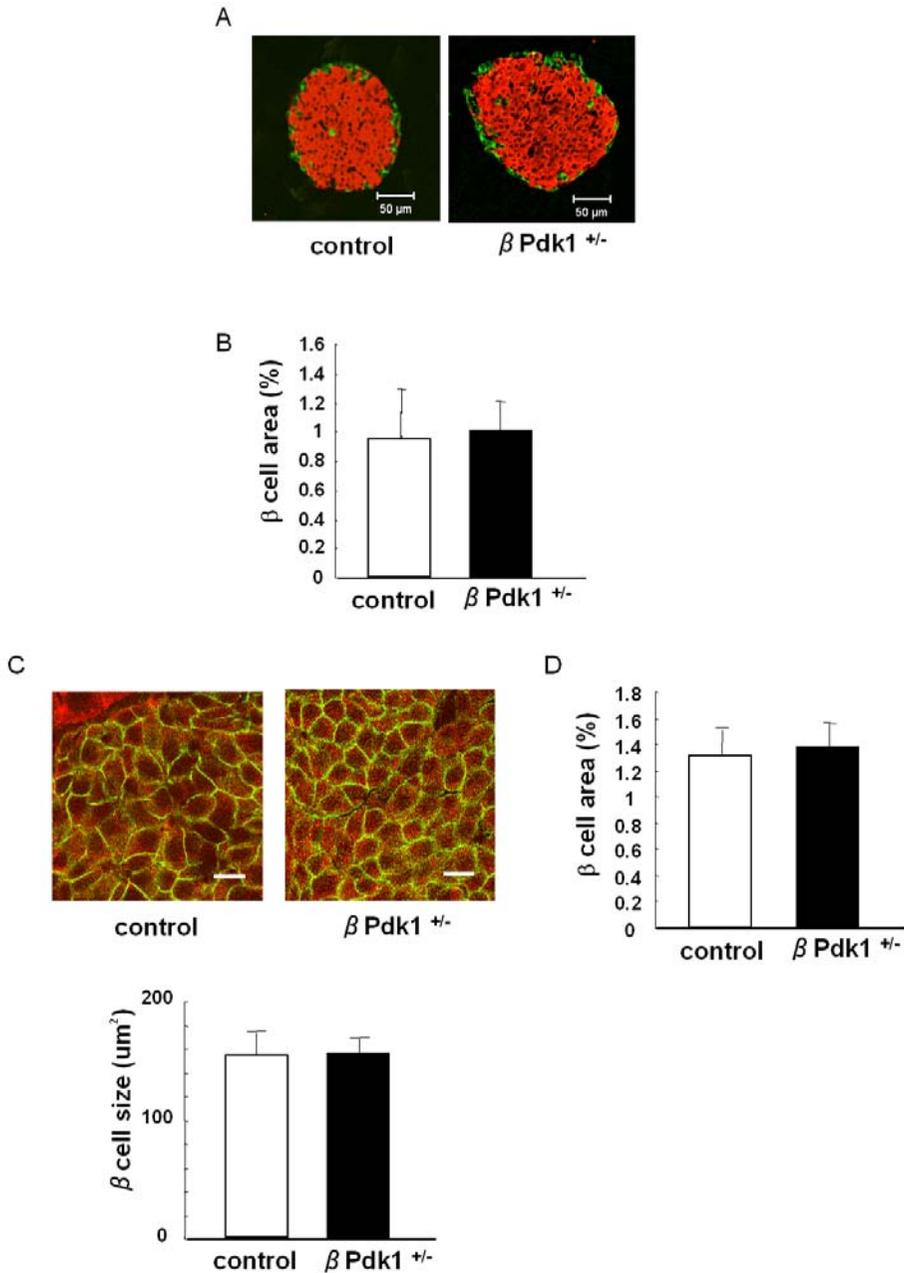
Figure 2 Effect of haploinsufficiency of PDK1 on islet morphology. (A) Immunostaining of pancreatic sections obtained from 24-week-old control and βPDK1^{+/−} mice with antibodies against insulin (red) and glucagon (green). Scale bars: 50 μm. (B) Quantification of β cell area as a percentage of the total pancreatic area in control and βPDK1^{+/−} mice. Data represented as means ± SEM of the values from 3 mice of each genotype. (C) Pancreatic sections were stained with antibodies against insulin (red) and β-catenin (green) to determine the size of individual β cells. Scale bars: 10 μm. (D) Quantification of β cell area as a percentage of the total pancreatic area in control and βPDK1^{+/−} mice fed a high-fat diet for 20 weeks. Data represented as means ± SEM of the values from 3 mice of each genotype.
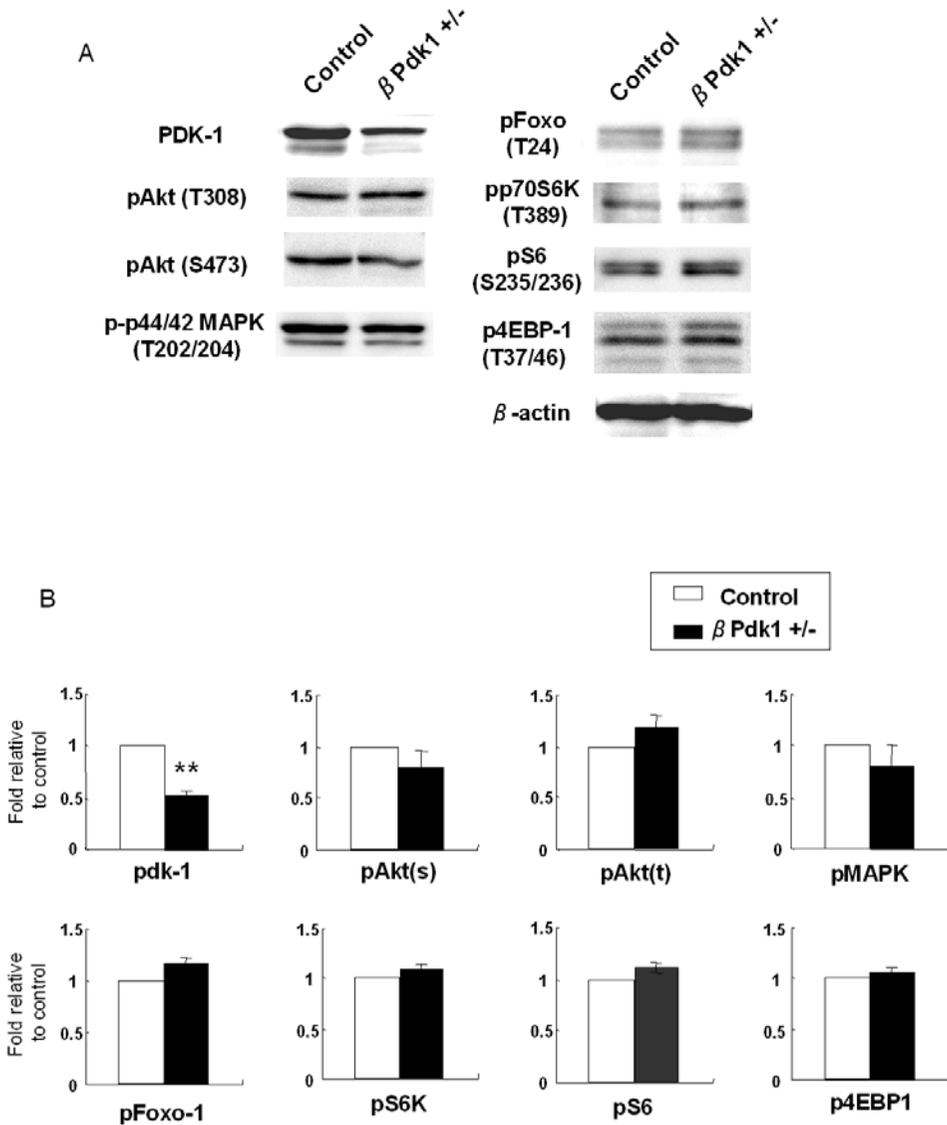
Figure 3 Effect of PDK1 haploinsufficiency on insulin signaling in pancreatic islets. Islets isolated from control and βPDK1^{+/−} mice were deprived of serum for 2 h and then incubated with 100 nM IGF-1 for 1 h. Next, the islets were lysed and subjected to immunoblot analysis with antibodies against phosphorylated (p) or total indicated proteins. (A) The results shown are representative of four independent experiments. (B) Quantification of phophorylated (p) or total indicated protein levels was performed by densitometric scanning and expressed as mean ± SEM of four independent experiments. ∗∗$P < 0.01$ versus the corresponding control value.

We have previously shown that mice deficient in PDK1, specifically in pancreatic β cells exhibit reduction in both size and number of β cells (5). However, mice deficient in PDK1, specifically in muscle exhibit a marked reduction in cardiac muscle mass as a result of a decrease in cell size rather than cell number (12). The pattern of regulation of cell size and cell number may thus be organ- or tissue-specific.

The hypothesis of our work based on the above mentioned results was that haploinsufficiency of PDK1 might induce reduction in β cell mass, particularly in a state of insulin resistance. However, the phenotype of the heterozygous PDK1 knockout mice was indistinguishable from that of the control mice because the reduced PDK1 levels in the βPDK1$^{+/-}$ mice might be insufficient to apparently affect the activation of Akt and p70 S6 kinase.

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