Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes

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1Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto; 2Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe; and 3Department of Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine, Kyoto, Japan

Okuno M, Minami K, Okumachi A, Miyawaki K, Yokoi N, Toyokuni S, Seino S. Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. Am J Physiol Endocrinol Metab 292: E158–E165, 2007. First published August 22, 2006; doi:10.1152/ajpendo.00180.2006.—We recently found that pancreatic acinar cells isolated from normal adult mouse can transdifferentiate into insulin-secreting cells in vitro. Using two different animal models of type 1 diabetes, we show here that insulin-secreting cells can also be generated from pancreatic acinar cells of rodents in the diabetic state with absolute insulin deficiency. When pancreatic acinar cells of streptozotocin-treated mice were cultured in suspension in the presence of epidermal growth factor and nicotinamide under low-serum condition, expressions of insulin genes gradually increased. In addition, expressions of other pancreatic hormones, including glucagon, somatostatin, and pancreatic polypeptide, were also induced. Analysis by the Cre/loxP-based direct cell lineage tracing system revealed that the newly made cells originated from amylase-expressing pancreatic acinar cells. Pancreatic secretion from the newly made cells was significantly stimulated by high glucose and other secretagogues. In addition, insulin-secreting cells were generated from pancreatic acinar cells of Komeda diabetes-prone rats, another animal model of type 1 diabetes. The present study demonstrates that insulin-secreting cells can be generated by transdifferentiation from pancreatic acinar cells of rodents in the diabetic state and further suggests that pancreatic acinar cells represent a potential source of autologous transplantable insulin-secreting cells for treatment of type 1 diabetes.

Materials and Methods

Diabetic animals. Diabetes was induced by intraperitoneal injection of 200 mg/kg STZ to 8- to 12-wk-old male C57Bl/6CrSlc mice or ROSA26 reporter mice, in which enhanced cyan fluorescent protein (eCFP) transgene is inserted into the ROSA26 locus with a floxed transcriptional stop sequence (R26R-eCFP) (26). Two days after injection of STZ, mice with blood glucose concentration above 19.4 mmol/l were used for isolation of exocrine pancreas. In some experiments, STZ-injected mice were maintained for 3 wk with daily administration of 2–4 U of NPH insulin (Novo Nordisk Pharma, Copenhagen, Denmark). We also used KDP rats, a diabetes-prone substrain of the Long-Evans Tokushima lean (LETL) rat (9, 34), with blood glucose concentration above 19.4 mmol/l–2 wk after the onset of hyperglycemia. All animal experiments were approved by the animal research committees of the Kyoto University Graduate School of Medicine and the Kobe University Graduate School of Medicine.

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Preparation and culture of pancreatic exocrine cells. Collagenase-digested pancreatic cells were subjected to Ficoll density gradient centrifugation, and the acinar cell-enriched fraction was recovered as a pellet. The absence of mature pancreatic islets in this fraction was confirmed by dithizone staining (22). The acinar cells-enriched fraction was cultured as reported (17). Briefly, the cells were plated onto sticky culture dishes in RPMI 1640 medium containing 10% fetal calf serum (FCS) for 6 – 8 h. The floating cells were then replated onto 2-methacryloyloxyethyl phosphorylcholine-treated Low-Cell-Binding dishes (Nalge Nunc International, Rochester, NY) in RPMI 1640 medium supplemented with 0.5% FCS, 20 ng/ml epidermal growth factor (EGF), and 10 mmol/l nicotinamide.

Immunocytochemistry. Cryostat sections were prepared from acinar-derived cell pellets fixed in 4% paraformaldehyde. The sections were blocked and permeabilized in phosphate-buffered saline containing 10% normal goat serum and 0.2% Tween 20. The primary and secondary antibodies used were the same as previously reported (17). Images were collected on a fluorescent microscope (Olympus, Tokyo, Japan) with a CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). Reverse transcriptase-polymerase chain reaction analysis. Total RNAs were isolated from acinar-derived cells, islets, MIN6-n9 cells (18), or INS-1 cells (1), using an RNeasy Mini Kit (Qiagen, Tokyo, Japan). After treatment with DNaseI (Qiagen), cDNA was prepared from 1 µg of total RNA by ReverTra Ace (Toyobo, Osaka, Japan), and subjected to PCR using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). The sequences of the primers, sizes of PCR products, and cycles for each pair are listed in either our previous study (17) or Table 1.

Quantitative real-time PCR was performed using either SYBR Green PCR master mix or TaqMan universal PCR master mix with an ABI-Prism 7300 sequence detector (Applied Biosystems). Measurement of the expression level of 18S ribosomal RNA (rRNA) was used as an internal control.

Cell lineage tracing. In R26R-eCFP reporter mice, an eCFP transgene is inserted into the ROSA26 locus and permanently expressed as an internal control. Induction of genes involved in pancreatic development and β-cell function. An insulin-deficient diabetic state was induced in mice by injection of STZ (200 mg/kg ip). Two days after the injection, the blood glucose levels of the mice were markedly elevated due to disruption of almost all of the pancreatic β-cells. Pancreatic acinar cells were isolated from mice with elevated blood glucose concentration (>19.4 mmol/l), and the absence of native pancreatic islets was confirmed by dithizone staining. We further characterized these isolated acinar cells by quantitative real-time RT-PCR (Fig. 1). Expressions of β-cell specific genes, including insulin-1 and -2, were almost absent, indicating that contamination of pancreatic β-cells in the starting material was negligible. We found reduced expression of amylase in the pancreatic acinar cells of STZ-injected mice compared with that in normal mice, suggesting that the diabetic state affected the function of exocrine pancreas (7, 11, 32). The cells were then cultured with 20 ng/ml EGF and 10 mmol/l nicotinamide in suspension. Morphologically, pancreatic acinar cells from STZ-injected mice formed spherical structures as seen in normal mice (17).

We next investigated expressions of other pancreatic genes during culture. Transcription factors necessary for the development of pancreas, such as Pdx1, NeuroD1, Foxa2, and HNF6, were induced in pancreatic acinar cells of STZ-injected mice (Fig. 2A). PGP9.5, a potential marker for endocrine progenitors (35), was induced. Genes of molecules that participate in glucose-induced insulin secretion in pancreatic β-cells, including glucokinase, SUR, Kir6.2, and Cav1.2, also were induced or increased by culture (Fig. 2B). Quantitative real-time RT-PCR analysis further confirmed induction of the genes associated with β-cell development and function (Fig. 2C). These results indicate acinar-to-endocrine transdifferentiation

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Table 1. List of gene-specific primers for RT-PCR analysis in rats

Statistical analysis. Data are expressed as means ± SE. The significance of differences between test groups was evaluated by t-test or by one-way analysis of variance followed by Scheffé’s test.

RESULTS

Induction of genes involved in pancreatic development and β-cell function. An insulin-deficient diabetic state was induced in mice by injection of STZ (200 mg/kg ip). Two days after the injection, the blood glucose levels of the mice were markedly elevated due to disruption of almost all of the pancreatic β-cells. Pancreatic acinar cells were isolated from mice with elevated blood glucose concentration (>19.4 mmol/l), and the absence of native pancreatic islets was confirmed by dithizone staining. We further characterized these isolated acinar cells by quantitative real-time RT-PCR (Fig. 1). Expressions of β-cell specific genes, including insulin-1 and -2, were almost absent, indicating that contamination of pancreatic β-cells in the starting material was negligible. We found reduced expression of amylase in the pancreatic acinar cells of STZ-injected mice compared with that in normal mice, suggesting that the diabetic state affected the function of exocrine pancreas (7, 11, 32). The cells were then cultured with 20 ng/ml EGF and 10 mmol/l nicotinamide in suspension. Morphologically, pancreatic acinar cells from STZ-injected mice formed spherical structures as seen in normal mice (17).

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in cells of STZ-injected mice similar to that found in normal mice.

**Insulin expression and its secretion in pancreatic acinar-derived cells of STZ-injected mice.** Expressions of insulin genes were evaluated by quantitative real-time RT-PCR. mRNA levels of both insulin-1 and insulin-2 genes were gradually increased by culture (Fig. 3A). In addition, insulin-positive cells were detected by immunostaining after culture (Fig. 3B).

We then examined insulin secretion in pancreatic acinar-derived cells of STZ-injected mice by the batch incubation method. When incubated in the presence of 30 mmol/l KCl, the cells exhibited a significant increase in insulin secretion over basal condition (3 mmol/l glucose; Fig. 3C), indicating Ca\(^{2+}\)-triggered exocytosis in the cells. Insulin secretion was also increased by glibenclamide (Fig. 3C), the sulfonylurea widely used in treatment of diabetes, indicating functional ATP-sensitive potassium channels.

Glucose stimulated insulin secretion from the pancreatic acinar-derived cells of STZ-injected mice in a concentration-dependent manner (Fig. 3D), indicating that the cells were glucose responsive. In addition, glucagon-like peptide-1 (GLP-1)-(7–36 amide) potentiated insulin secretion (Fig. 3D), indicating the cAMP-mediated potentiation system was also present in the cells. Carbachol, a synthetic acetylcholine derivative that mobilizes intracellular Ca\(^{2+}\) through muscarinic receptors, stimulated insulin secretion (Fig. 3C), indicating that the phosphatidylinositol signaling system is involved in insulin secretion in these cells. It was noted that no insulin secretion was detected on the day of isolation (Fig. 3C). These results demonstrate that glucose-responsive insulin-secreting cells can be generated from pancreatic acinar cells of β-cell-deficient diabetic mice.

**Transdifferentiation of pancreatic acinar cells of mice with chronic hyperglycemia.** We also attempted to induce insulin-secreting cells from pancreatic acinar cells of mice with chronic hyperglycemia. STZ-injected mice were maintained for 3 wk with daily administration of NPH insulin (2–4 U). By this treatment, the mice were able to survive despite hyperglycemia (~11–28 mmol/l). We found that Ptf1a expression was downregulated by chronic hyperglycemia (Fig. 1). However, pancreatic acinar cells obtained from mice with chronic hyperglycemia began to express β-cell-specific genes by culture (Fig. 4A), and the newly generated cells could secrete insulin in response to high KCl and glucose (Fig. 4B). These results demonstrate that pancreatic acinar cells retain plasticity in their differentiation capacity even in a chronic diabetic state.

**Cell lineage tracing of pancreatic acinar cells of STZ-injected mice.** As expressions of pancreatic acinar cell marker genes (amylase and elastase) were decreased, expressions of insulin genes and cytokeratin (CK)19 were induced by the culture (Fig. 5A). We have previously established by direct cell lineage tracing that pancreatic acinar cells of normal mice can transdifferentiate into insulin-secreting cells and CK-expressing cells (17). In this study, we utilized STZ-injected R26R-eCFP reporter mice for tracing. As was found in normal mice (17), cells positive for both insulin and eCFP were found in this
In addition to insulin, expressions of the STZ-injected mice. CK/eCFP double-positive cells also cells were transdifferentiated from pancreatic acinar cells of culture system (Fig. 5A). Cells positive for eCFP and glucagon, somatostatin, or other pancreatic hormones were also induced by culture (Fig. 5B). pancreatic polypeptide (PP) were detected (Fig. 5C), indicating that these cells originated from pancreatic acinar cells. These results demonstrate that pancreatic acinar cells of β-cell-deficient diabetic mice can transdifferentiate into all types of pancreatic endocrine cells as well as ductal cells.

**Generation of insulin-secreting cells from KDP rats.** We then investigated to find whether pancreatic acinar cells of spontaneously diabetic animals could transdifferentiate into insulin-secreting cells. The KDP rat, which was established as a diabetes-prone substrain of the LETL rat, is a model of type 1 diabetes (9, 34). The phenotypic features of the KDP rat include autoimmune destruction of the pancreatic β-cells, and about 80% of them develop diabetes within 220 days of age (9). We used diabetic KDP rats with blood glucose concentrations above 19.4 mmol/l. No dithizone-stained islets or fragmented islets were found in the acinar-enriched fraction from KDP rats, indicating the absence of native pancreatic β-cells in the fraction. RT-PCR analysis revealed that both insulin-1 and insulin-2 genes were induced by the culture in the pancreatic acinar cells of KDP
Fig. 3. Insulin expression and secretion in pancreatic acinar-derived cells of STZ-injected mice. A: quantitative real-time PCR analysis for insulin genes using SYBR Green PCR master mix. Measurement of the expression level of 18s rRNA was used as an internal control. Insulin expression in the culture was gradually increased. B: immunostaining for insulin. Insulin-producing cells (green) were generated from β-cell-deficient mice 6 days after culture. Nuclei were stained with DAPI (blue). Scale bar, 20 μm. C and D: insulin secretion in acinar-derived cells from STZ-treated diabetic mice. Insulin secretion was measured as accumulation during 60-min incubation. The secretion at 3 mmol/l glucose (G3) represents basal secretion. Addition of 30 mmol/l KCl, 0.1 μmol/l glibenclamide (Glib), or 0.1 mmol/l carbachol (Cch) increased insulin secretion. (C). Glucose stimulated insulin secretion in a concentration-dependent manner (G3, 3 mmol/l; G10, 10 mmol/l; G20, 20 mmol/l). Glucagon-like peptide-1 (GLP-1)-(7–36 amide) (100 mmol/l) potentiated insulin secretion in the presence of glucose (D). The amount of secreted insulin at 3 mmol/l glucose was 714 ± 143 pg/mg protein. Data are means ± SE of 3–5 independent experiments.

rats as well as in those of normal Wistar rats (Fig. 6, A and B). In addition, the expression profiles of other pancreatic genes in pancreatic acinar-derived cells of both normal Wistar rats and KDP rats became similar to that of the rat insulinoma cell line INS-1 (1) (Fig. 6, A and B). We then measured insulin secretion in pancreatic acinar-derived cells of KDP rats by the batch incubation method. Although glucose-induced insulin secretion was not observed, a high concentration of KCl clearly stimulated insulin secretion (Fig. 6C). These results demonstrate that insulin-secreting cells can be transdifferentiated from pancreatic acinar cells of spontaneously diabetic animals.

Fig. 4. Transdifferentiation of insulin-secreting cells from pancreatic acinar cells of mice with chronic hyperglycemia. A: RT-PCR analysis of genes involved in pancreatic development and β-cell function. Pancreatic β-cell-specific genes were induced by culture. B: insulin secretion. Secretion at 3 mmol/l glucose (G3) represents basal secretion. Addition of 30 mmol/l KCl or 20 mmol/l glucose increased insulin secretion.
DISCUSSION

The present study establishes that pancreatic acinar-to-endocrine transdifferentiation resulting in generation of insulin-secreting cells can occur in β-cell deficient diabetic animals in vitro. Diabetes is characterized by chronic hyperglycemia due to relative or absolute deficiency of insulin action (5). In addition, metabolic disorders in lipids, amino acids, and other nutrients are often found in diabetic patients (4, 27). These abnormalities may influence the cellular function of a variety of tissues (19). Pancreatic exocrine function also is known to be impaired in diabetes (7, 11, 14, 32). We found reduced amylase expression in acinar cells of STZ-injected mice. Similar observations were reported in STZ-injected rats, in which the mRNA level of pancreatic amylase was decreased (10, 13). However, this reduction of amylase expression exerted only a small, if any, effect on transdifferentiation capacity of acinar cells.

Insulin was not detected in pancreatic acinar cell-enriched fractions at the protein level on the day of isolation. Insulin mRNA was detected in pancreatic acinar cell fractions of STZ-injected mice, although the amount was extremely small. This raises the possibility that increased expressions of endocrine-associated genes result from enrichment and/or proliferation of contaminated endocrine cells by culture. However, this can be ruled out by the following considerations: 1) binucleated insulin-positive cells were often found (Fig. 4B); 2) almost

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**Fig. 5.** Generation of endocrine cells from pancreatic acinar cells of STZ-injected mice. **A:** RT-PCR analysis of genes associated with endocrine pancreas. Both insulin-1 and insulin-2 genes were induced in pancreatic acinar cells of normal Wistar rats (A) and KDP rats (B) by the culture. Expression patterns of other pancreatic genes became similar to that of rat insulinoma cell line INS-1 by the culture in pancreatic acinar-derived cells from normal Wistar (A) and KDP rats (B). **C:** insulin secretion from pancreatic acinar-derived cells of KDP rats. Insulin secretion was increased significantly by addition of 30 mmol/l KCl. The amount of secreted insulin at 3 mmol/l glucose was 97 ± 56 pg/mg protein.

**Fig. 6.** Generation of insulin-secreting cells from Komeda diabetes-prone (KDP) rats. A and B: RT-PCR analysis of genes associated with endocrine pancreas. Both insulin-1 and insulin-2 genes were induced in pancreatic acinar cells of normal Wistar rats (A) and KDP rats (B) by the culture. Expression patterns of other pancreatic genes became similar to that of rat insulinoma cell line INS-1 by the culture in pancreatic acinar-derived cells from normal Wistar (A) and KDP rats (B).
all of the insulin-positive cells expressed PGP9.5 (17); 3) cell proliferation was rarely detected, especially in insulin-positive cells, in this culture system (17); and 4) the expression of pancreatic exocrine specific transcription factor Mist1 showed no significant change by the culture (Fig. 2). Although transdifferentiation of pancreatic acinar cells into endocrine cells occurs in our culture system, because the transdifferentiated insulin-secreting cells have features not found in native β-cells (two nuclei, expressions of PGP9.5 and Mist1), these cells clearly are not fully differentiated endocrine cells. The low insulin production may reflect this immaturity. In addition, we could not detect Pax4 expression at any time point during culture. Since Pax4 has been shown to be essential in development of pancreatic β-cells (25), the absence of Pax4 might indicate that the newly made insulin-secreting cells are incompletely differentiated. Alternatively, the process of transdifferentiation of insulin-secreting cells from acinar cells might differ from that in normal β-cell development.

In the present study, we found that all types of pancreatic endocrine cells can be generated from pancreatic acinar cells by our culture system. We provide clear evidence by direct cell lineage tracing that adult mouse pancreatic acinar cells can differentiate into cells expressing insulin, glucagon, somatostatin, PP, and CK. It has been reported that pancreatic acinar cells from rat can convert into liver cells (12). We also detected mRNAs of albumin and α-fetoprotein in our system (data not shown). These findings indicate that adult pancreatic acinar cells (or dedifferentiated acinar cells) possess multipotentiality in differentiation capacity.

We also found that pancreatic acinar cells of KDP rats undergo transdifferentiation by culture with EGF and nicotine amide. Although the STZ-injected mice diabetes model represents chemically induced acute hyperglycemia with hypoinsulinemia, the diabetic state of KDP rats is genetically established and develops chronically (9). KDP rats show autoimmune destruction of the pancreatic β-cells (9), as is found in human type 1 diabetes. Thus our success in generating insulin-secreting cells from pancreatic acinar cells of KDP rats is of special significance regarding application of these techniques to human subjects in the future. However, the insulin production and secretory responses are even lower in cells from KDP rats than from STZ-injected mice. Apparently, genes involved in glucose sensing (GLUT2 and glucokinase) and metabolism-secretion coupling (ATP-sensitive potassium channels and voltage-dependent calcium channels) are insufficiently induced. Further studies are required to investigate differences between STZ-injected mice and KDP rats in the transdifferentiation capacity of pancreatic acinar cells.

In conclusion, our data demonstrate that insulin-secreting cells can be generated from pancreatic acinar cells of insulin-deficient diabetic animals in vitro. Thus the present study is an important first step toward treatment of type 1 diabetes by autologous transplantation using pancreatic acinar-derived insulin-secreting cells.

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