Pancreatic acinar-to-beta cell transdifferentiation in vitro

Minami, Kohtaro

Seino, Susumu

Pancreatic acinar-to-beta cell transdifferentiation *in vitro*

Kohtaro Minami[1,2] and Susumu Seino[1, 2,3]

[1]*Division of Cellular and Molecular Medicine, Department of Physiology and Cell Biology, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan*

[2]*Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, 54 Shogoinkawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan*

[3]*Division of Diabetes, Metabolism, and Endocrinology, Department of Internal Medicine Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan*

TABLE OF CONTENTS

1. ABSTRACT

Pancreatic beta-cell mass in adults can be maintained by several mechanisms, including self-replication of beta-cells, neogenesis from unidentified stem/progenitor cells, and transdifferentiation from non-beta-cells in pancreas such as pancreatic acinar and duct cells. Although accumulating evidence indicates that proliferation of pre-existing beta-cells is the major mechanism of the maintenance of postnatal beta-cell mass, new beta-cells can be generated from non-beta-cells under certain conditions *in vitro*. We have recently shown directly by Cre/loxP-based cell lineage tracing that adult mouse pancreatic acinar cells can be transdifferentiated into insulin-secreting cells *in vitro*. These newly made cells secrete insulin in response to glucose and other secretagogues, but their secretory capacity is still low compared to that of native beta-cells. To improve the efficiency of generation of insulin-secreting cells from non-beta cells, it is critical to understand the molecular mechanism of such transdifferentiation. Since pancreatic acinar cells are the most abundant cell type in the pancreas, their utilization as a source of surrogate beta-cells is an intriguing approach to cell replacement therapy for type 1 diabetes. This review focuses on current knowledge of the regeneration of pancreatic beta-cells and transdifferentiation of pancreatic acinar-cells into insulin-secreting cells.

2. INTRODUCTION

Insulin has been in general use in the treatment of diabetes with absolute insulin deficiency since its discovery. However, normal pancreatic beta-cells continually adjust insulin secretion in response to varying blood glucose levels, while daily exogenous insulin administration cannot maintain blood glucose levels within the narrow physiological range that protects from development of the various diabetic complications, and more precise administration is impracticable. Thus, transplantation of insulin-secreting cells generated from stem (or progenitor) cells is a most promising therapeutic approach to treatment of insulin-deficient diabetes (1-3). Although it has been reported that embryonic stem (ES) cells can be manipulated to produce insulin (4-7), there are several obstacles to their clinical use. Transplantation of ES cell derivatives into human recipients can result in the formation of ES cell-derived tumors (8). Ethical problems arise in the acquisition of human ES cells (9). In addition, it has been suggested that both the production and the release of insulin in such manipulated ES cells may be abnormal (10,11).

On the other hand, recent studies have shown that insulin-secreting cells can be generated *in vitro* from adult non-beta-cells including mouse and human pancreatic duct cells (12-14), rat hepatic oval cells (15), and mouse bone marrow cells (16). Pancreatic acinar cells represent the most abundant cell type in the pancreas. In addition, a large number of pancreatic acinar cells can readily be obtained as a byproduct of islet transplantation. Considering that all types of pancreatic cells originate from the same stem cells (17), the generation of transplantable insulin-secreting beta-cells from pancreatic acinar cells would seem to be feasible. Indeed, we and others have shown that isolated pancreatic acinar cells can convert into insulin-secreting cells under certain conditions (18-20). However, full differentiation into beta-cells has not been achieved, and little is known of the signaling pathways involved in the regeneration of pancreatic beta-cells. Thus, clarification of the mechanism of pancreatic beta-cell regeneration should be important for not only deepening our understanding of the physiology of pancreatic islets but also providing a basis for strategy of cell replacement therapy for diabetes mellitus.

3. DEVELOPMENTAL ASPECTS OF PANCREATIC CELLS

3.1. Early development of the pancreas

The pancreas is an organ comprising two distinct populations of cells, exocrine cells producing and secreting digestive enzymes through the duct system into the gut and endocrine cells producing and secreting hormones into the bloodstream.   Although each of the differentiated pancreatic cell types possesses a different function, all pancreatic cells are differentiated from common progenitor cells in primitive gut.   In mouse, formation of the pancreas becomes evident at embryonic day 8.5-9.0 immediately after closure of the anterior endoderm (21).   The pancreas derives from two distinct buds on the foregut.   One anlage arises dorsally in the duodenal part on the foregut and the other is formed ventral to the hepatic endoderm, which then fuse to form the pancreas.   However, little is known of the molecular mechanisms of early specification of the gut tube in the formation of the pancreatic buds, although inductive signals from adjacent germ layers are thought to be involved (17,22).   It has been suggested that signals from the notochord and endothelium specify the location of the pancreatic epithelium, while those from mesenchyme regulate the size of the pancreas (21).

3.2. Cell signals involved in pancreas development

Notch signaling has been thought to play a critical role in cell type specification of the pancreas (23,24). The Notch pathway acts on binary cell fate by lateral inhibition, which is required in the developing pancreas to maintain the progenitor cell population (25).   The Notch receptor is a single transmembrane protein composed of extracellular, transmembrane, and intracellular domains. Notch ligand Delta/Serrate/Lag2 (DSL) family proteins on neighboring cells activate the Notch pathway, leading to proteolysis that releases the Notch intracellular domain (NICD) and results in the expression of Hairy and Enhancer-of-split protein 1 (Hes1), a downstream molecule in the Notch signaling pathway (23). Suppression of Notch signaling depletes the progenitor pool by precocious differentiation and upregulation of neurogenin 3 (Ngn3) (26,27).   Conversely, activation of Notch signaling prevents endocrine differentiation (24,28).   Thus, the Notch signaling pathway is involved in the regulation of cell differentiation and proliferation in developing pancreas.

Interaction between epithelial and mesenchymal cells also participates in pancreas development.   For example, fibroblast growth factor 10 (FGF10) released from mesenchyme plays an important role in the maintenance Pdx1-positive epithelial progenitors in the pancreas (29).   While lack of FGF10 expression results in severe growth retardation of the pancreas (29), ectopic expression of FGF10 by pancreas-duodenum homeobox 1 (Pdx1) promoter causes abrogation of pancreatic cell differentiation and sustained proliferation of pancreatic progenitor cells (30,31). Furthermore, interplay between FGF10 and Notch signaling has been reported (32,33).   Epidermal growth factor (EGF) signaling also may be involved in pancreas development. EGF receptor family members (ErbBs) are known to be expressed in developing pancreas (33), and mutation in the ErbB3 receptor impairs the development of pancreas (34). EGF also was shown to promote proliferation of mesenchyme-depleted rat pancreatic epithelium *in vitro* (35). Moreover, transforming growth factor (TGF)-beta signaling is important for growth and differentiation of pancreatic cells. The TGF-beta superfamily consists of TGF-beta, bone morphogenetic proteins (BMPs), and activins, which bind to two types of serine/threonine kinase receptors.   A dominant-negative form of the type II receptor of TGF-beta increases

proliferation and impairs differentiation of exocrine pancreas (46).   In addition, hyperplasia of pancreatic islets has been reported in transgenic mice expressing a dominant-negative activin receptor (37).   Furthermore, expression of BMPs is detected in developing pancreas (38-40), suggesting their roles in pancreas development.

3.3. Transcription factors in developing pancreas

Pancreatic progenitors within the foregut epithelium can be discerned by the expression of Pdx1, which appears at e8.5 in the foregut endoderm and is expressed throughout the pancreatic epithelium at e9.5 (41).   Pdx1 expression is lost in mature acinar and ductal cells in adult pancreas, but is maintained in differentiated pancreatic beta-cells.   In Pdx1-deficient mice, both pancreatic buds form initially, but are arrested at a very early stage of development, resulting in agenesis of the pancreas (42).   Thus, Pdx1 is essential for the growth of pancreatic buds and is considered to be the master regulator of pancreas development.   Pancreas transcription factor 1a (Ptf1a) is also expressed throughout the developing pancreas beginning at e9.5 (43), and has been shown to be required for the growth of pancreatic buds (44).   Ptf1a is maintained in differentiated pancreatic acinar cells, while it is down-regulated in endocrine and duct cells.   In addition, forkhead box a2 (Foxa2) is known to activate Pdx1 expression (45).   Moreover, the basic-helix-loop-helix (bHLH) transcription factor Ngn3, a downstream target of hepatocyte nuclear factor 6 (HNF6), is expressed in pancreatic endocrine progenitor cells, and has been shown to be required for endocrine differentiation. Ngn3-deficient mice lack endocrine cells completely (46). A lineage tracing study by Gu et al. has shown that Ngn3-expressing cells give rise to all four endocrine cell types (47).   Ngn3 induces the expression of neurogenic differentiation 1 (NeuroD1) and paired box gene 4 (Pax4), both of which are essential for beta-cell function.   However, the expression of Ngn3 itself is lost in differentiated endocrine cells.   NeuroD1 induces the expression of genes characteristic of pancreatic beta-cells, including insulin and the molecules required for regulated excytosis (48). Collombat et al. reported that Aristaless-related homeobox (Arx) plays an important role in the specification of alpha-versus beta-cells in a complementary fashion to Pax4 (49).

4. MAINTENANCE OF ADULT PANCREATIC BETA-CELLS

4.1. Neogenesis and regeneration in the pancreas

In general, adult tissues and organs are thought to be maintained by the stem cell system, in which stem cells having the potential to self-renew give rise to progenitor cells, which then proliferate and differentiate into the several types of functionally differentiated cells (Figure 1).   The primary role of adult (tissue-specific) stem cells is to maintain and repair the tissue in which they reside.   The best-known example of the stem cell system is the generation of blood cells.   Hematopoietic stem cells (HSCs) reside in bone marrow in adult and have multipotency to differentiate into all types of mature blood cells (50,51).   HSCs also have the potential to self-renew, although whether or not asymmetric cell division, in which of one daughter cell that remains a stem cell and one daughter cell that differentiates is generated, occurs during self-renewal is not known (50,51).   HSCs give rise to committed precursor cells such as proerythroblasts, myeloblasts, lymphoblasts, monoblasts, and megakaryoblasts, which differentiate into mature blood cells (50,51).   A similar stem cell system is found in small intestine, which undergoes constant renewal and differentiation from stem cells throughout the lifetime (52).   In contrast to blood cells and intestinal epithelial cells that are required to renew everyday, cells in the pancreas seem to be static.   However, in certain situations including pathological conditions and

4

experimental models, remodeling of the pancreas occurs. In transgenic mice expressing interferon (IFN)-gamma specifically in pancreatic beta-cells, a dramatic proliferation of pancreatic ductal cells and the appearance of primitive endocrine cells and their subsequent differentiation into endocrine cells has been reported (53). During regeneration, transitional intermediate cells expressing both carbonic anhydrase II and amylase (54), and bearing both endocrine and exocrine granules (55) appear. The authors speculate from these findings that pancreatic duct cells represent facultative progenitors in adult pancreas. However, their results also suggest that pancreatic acinar cells give rise to intermediate cells, which have characteristics of pancreatic duct cells, and then differentiate into endocrine cells. It has been reported that overexpression of TGF-alpha induces expansion of Pdx-1-expressing ductal epithelium in the pancreas, and that focal areas of islet neogenesis are observed (56). Since pancreatic acinar cells isolated from TGF-alpha transgenic mice convert into ductal cells (57,58), the expanded pancreatic ductal cells expressing Pdx1 in these mice may well be derived from pancreatic acinar cells.

In addition to these genetically engineered mice, some pancreatic injury models have been shown to exhibit pancreas regeneration. After ligation of the pancreatic duct in rats, replacement of exocrine acini by duct-like structures is observed (59). This acinoductal metaplasia has been thought to be at least in part due to transdifferentiation of amylase-positive pancreatic acinar cells into amylase-negative and cytokeratin-positive duct-like cells (60). By treating the rats with dexamethason to inhibit loss of amylase expression, transitional cells co-expressing amylase and cytokeratin 20 (CK20) were detected (60), supporting the notion of acinar-to-ductal transdifferentiation. Moreover, insulin-positive cells that also express amylase were found, indicating acinar-to-endocrine transdifferentiation.

The beta-cell toxin streptozotocin (STZ) is known to cause rapid and massive beta-cell death. In some studies, it has been found that insulin-positive cells reappear after the loss of pancreatic beta-cells by STZ treatment (61,62). During regeneration, somatostatin/Pdx1-double positive cells, which subsequently differentiate into somatostatin/insulin/Pdx1-positive cells, are found in pancreatic islets. Exogenous administration of insulin enhances this beta-cell differentiation and maturation. These findings suggest that adult pancreatic islets contain a progenitor cell population that can be differentiated into insulin-positive beta-cells following islet injury. Thus, although the adult stem cells responsible for the maintenace of the pancreas have not yet been identified, these results sugget the existence of cells with stem cell-like capacity in adult pancreas. The potential candidates include pancreatic islets (62-64), duct cells (65), and acinar cells (18-20).

It is noteworthy that all of these models of regeneration involve injury of the pancreas. Thus, while the signals inducing or initiating beta-cell regeneration remain largely unknown, growth factors and inflammatory cytokines are implicated in the process. It has been reported that the Notch pathway is involved in pancreatic acinar-to-ductal transdifferentiation in TGF-alpha transgenic mice (57). When gamma-secretase inhibitor, which prevents cleavage of Notch receptors, was applied to isolated pancreatic acinar cells from TGF-alpha transgenic mice, induction of Hes1 expression was eliminated and formation of duct-like structures was reduced. The EGF receptor kinase inhibitor AG1478 completely abolished induction of acinar-to-ductal transdifferentiation by TGF-alpha, but had no effect on transdifferentiation by the activated intercellular domain of Notch1. Thus, activation of the Notch pathway is a downstream mediator of EGF receptor signaling in pancreatic acinar-to-ductal transdifferentiation (57).

4.2. Pancreatic tissue-specific stem/progenitor cells

Many investigators have attempted to identify stem/progenitor cells in adult pancreas. Zulewski et al. showed that cells expressing the neural stem cell marker nestin occur in human and rat pancreatic islets, and that these cells can be isolated and cultured for a long time (63). It has been shown that cultured nestin-positive cells can be differentiated into insulin-producing cells (63,64), and that such cells from human fetal pancreas when transplanted can be expanded and differentiated into islet-like cell clusters, which can reverse hyperglycemia in diabetic mice (66). Clonal identification of multipotent precursors from adult mouse pancreas has recently been reported (67). These candidate progenitor cells proliferate in the serum-free conditions used for neural stem cell (NSC) culture, and form spherical cell clusters like NSCs by floating culture. The spheroids containing progenitor cells can be derived from both pancreatic islets and duct cell populations. Although the cells were generated from both nestin-positive and nestin-negative fractions, they all expressed nestin during the expansion period. Interestingly, the cells in the spherical clusters show characteristics of both pancreatic and neural precursors. Moreover, the potential progenitors give rise to multiple types of neural cells including neurons and glial cells, and also differentiate into insulin-producing (beta) cells, glucagon-producing (alpha) cells, and somatostatin-producing (delta) cells. The insulin-producing cells derived from these progenitors are glucose-competent in terms of Ca^{2+} responsiveness and insulin secretion. The pancreatic islet-like cells are generated from the spheroids by culture onto Matrigel matrix-coated plates without the addition of mitogens. However, since the Matrigel basement membrane contains various growth factors, the signals responsible for islet cell generation could not be identified. Suzuki et al. also reported isolation of pancreatic progenitor cells from adult mouse by using fluorescence-activated cell sorting (FACS) (68). The isolated cells express cMet, the receptors for hepatocyte growth factor (HGF), but do not express hematopoietic and vascular endothelial antigens such as CD45, TER119, c-Kit, and Flk-1. Thus, HGF/c-Met signaling may play an important role in the maintenance of these progenitor-like cells. The cells formed clonal colonies *in vitro* and differentiated into multiple pancreatic lineages from single cells. As glucagon-like peptide-1 (GLP-1) was used in that study, GLP-1-mediated cAMP signaling may well participate in the differentiation of the candidate progenitor cells into mature pancreatic cells. However, no functional analysis has been done for the differentiated pancreatic cells induced from these candidate progenitors. Considered together, these findings suggest that while stem/progenitor-like cells can be obtained from adult pancreas, it is not yet clear that such isolated stem/progenitor-like cells have full potential to differentiate into native pancreatic beta-cells and function as stem/progenitors in the pancreas *in vivo*. In fact, a recent study demonstrated that the size of the mouse pancreas, unlike that of the liver, is limited by the number of embryonic progenitor cells (69), suggesting that stem/progenitor cells, if they do exist, may not improve function in adult pancreas. Thus, both the existence and the nature of stem/progenitor cells in adult pancreas are yet to be established.

4.3. Expansion of pre-existing beta-cells

It is known that pancreatic beta-cell mass expands through adulthood (70-73). Although histological analysis has demonstrated that neogenesis or regeneration of pancreatic beta-cells occurs in certain conditions, the cellular origin of the new beta-cells has not been demonstrated. Recent studies using genetic cell lineage tracing or other sophisticated methods suggest that adult pancreatic beta-cells are not derived from non-beta-cells (74-76). By using genetic cell lineage tracing, Dor et al. demonstrated that adult pancreatic beta-cells in mice are maintained

predominantly by self-replication of pre-existing beta-cells (74). They labeled pancreatic beta-cells selectively with human alkaline phosphatase by Cre-loxP-based conditional recombination in adult pancreas and chased the fate of pre-existing beta-cells. In this system, when new beta-cells in adult pancreas are generated from non-beta-cells such as stem/progenitor cells, the frequency of the labeled beta-cells should decrease after a chase period. The results indicated that the labeling frequency of the beta-cells remained unchanged, indicating that new beta-cells were generated primarily from pre-existing beta-cells. They conclude that terminally differentiated beta-cells retain proliferative capacity and cast doubt on a significant role for adult stem cells in beta-cell replenishment (74). Georgia and Bhushan reported that during neonatal development, cyclin D2 expression in the endocrine pancreas coincided with the replication of endocrine cells and a massive increase in islet mass (75). Cyclin D2 is not required for exocrine and ductal cell proliferation but is required for replication of endocrine cells. In cyclin D2$^{-/-}$ mice, pancreatic islets are much smaller and beta-cell mass is reduced to 25% in comparison with wild type mice. Thus, cyclin D2 plays a key role in beta-cell replication, which may be the primary mechanism for maintaining postnatal beta-cell mass (75). Furthermore, Teta et al. showed by using a DNA analog-based lineage-tracing technique that unlike gastrointestinal and skin epithelia, specialized progenitors do not contribute to adult beta-cell mass, not even during acute beta-cell regeneration (76). Instead, adult beta-cells exhibit equal proliferation potential, and expand from within a vast and uniform pool of mature beta-cells (77). Thus, it is likely that pancreatic beta-cell mass is maintained primarily by self-replication of pre-existing beta-cells in adult mice, although the existence of pancreatic tissue-specific stem/progenitor cells cannot be excluded.

5. TRANSDIFFERENTIATION IN THE PANCREAS

5.1. Plasticity of pancreatic cells

Although it has been thought that terminally differentiated cells do not change their phenotype, accumulating evidence suggests that phenotypic plasticity is retained in differentiated cells. Transdifferentiation, the conversion of one already differentiated cell type to another, is a paradigm of phenotypic plasticity in adult cells. In general, such phenotypic change occurs in tissues with chronic damage and in tissue regeneration (78). The pancreas is an organ in which metaplasia, a pathological state involving transdifferentiation (79,80), frequently occurs. For example, hepatocyte-like cells appear in human pancreatic cancer in some cases (81), and experimental conditions such as copper depletion can lead to the development of pancreatic hepatocytes in rodents (82,83). Moreover, metaplastic hepatocytes in ciprofibrate-treated rat pancreas have been shown to originate from pancreatic exocrine acinar cells (84). Pancreatic acinar cells also can convert into ductal cells, and direct evidence for acinar-to-ductal transdifferentiation has been provided both *in vitro* (20,58) and *in vivo* (85,86). In addition, transdifferentiation of insulin-secreting cells from pancreatic exocrine cells has been demonstrated *in vitro* (19,20), and cell lineage tracing has revealed the origin of the newly made insulin-secreting cells to be pancreatic acinar cells (20,87).

The signaling mechanisms of transdifferentiation of pancreatic acinar cells are not fully understood. Although, EGF, Notch, and/or leukemia inhibitory factor (LIF)/(signal transducer and activator of transcription (STAT)) signals are thought to be involved in the process (19,20,57,58), their precise roles are not known. In addition, putative progenitor cell markers such as nestin (58) and protein gene product 9.5 (PGP9.5) (20,88) have been detected in transdifferentiating pancreatic acinar cells, suggesting that dedifferentiated intermediate cells were generated in the

7

course of the transdifferentiation, as seen in lens or tail regeneration in amphibia (89,90). However, such dedifferentiated states are transient, and the intermediate cells have not been characterized. Thus, the details of the process of transdifferentiation of pancreatic cells are largely unknown at present.

5.2. Beta-cell transdifferentiation

Differentiated pancreatic beta-cells were thought to be static, but several studies have suggested that beta-cells transdifferentiate into exocrine and duct-like cells *in vitro* (91,92). Yuan et al. reported that cultivation of purified human islets in collagen gel matrix resulted in transformation of islet cells into duct-like structures without insulin expression (91). In a study by Scmied et al., when purified human pancreatic islets were cultured, endocrine cells gradually transdifferentiated into ductal, acinar, and intermediary cells. After a long period of culture (> 60 days), the endocrine-derived cells were converted into undifferentiated cells expressing neuron-specific enolase, chromogranin A, laminin, vimentin, CK7 and CK19, alpha-1-antitrypsin, TGF-alpha, and EGF receptor (92). Thus, human islet cells possess plasticity to change their differentiated phenotype *in vitro*. However, a recent study using genetic cell lineage tracing has found that pre-existing pancreatic beta-cells do not contribute to regeneration or metaplasia in pancreatitis *in vivo* (93).

5.3. Acinar cell transdifferentiation

There are many reports regarding phenotypic plasticity of pancreatic acinar cells. As described above, studies of IFN-gamma transgenic mice (53,54), TGF-alpha transgenic mice (56-58), and duct ligation models (59) suggest that pancreatic exocrine acinar cells transdifferentiate into ductal and endocrine cells after inflammation. Transdifferentiation of pancreatic acinar cells into ductal cells (58) and insulin-secreting cells (20) has been demonstrated *in vitro* under certain conditions without gene transfer. In addition, a rat pancreatic tumor-derived cell line AR42J, which has features of pancreatic acinar cells, has been shown to differentiate into insulin-secreting cells in the presence of betacellulin and activin A (94). In contrast, recent studies have failed to detect transdifferentiation of pancreatic acinar cells either in normal or pathological conditions *in vivo* (93,95). Desai et al. have shown by genetic cell lineage tracing that pancreatic acinar cells contribute only to regeneration of the acinar cells but neither beta-cells nor ductal cells *in vivo* (95). However, these studies do not exclude the possibility of *in vitro* or *ex vivo* transdifferentiation of pancreatic acinar cells into pancreatic beta-cells or ductal cells. It should be noted that direct evidence *in vivo* has been provided for acinar-to-ductal transdifferentiation in transgenic mice persistently expressing Pdx1 in pancreatic acinar cells (85) and mice with cerulein-induced pancreatitis (86), demonstrating the plasticity of pancreatic acinar cells in their differentiation capacity. Clarification of the mechanism of transdifferentiation is required to explain these discrepancies**.**

6. PANCREATIC ACINAR-TO-ENDOCRINE TRANSDIFFERENTIATION

6.1. Generation of insulin-secreting cells from pancreatic acinar cells

In vitro generation of insulin-secreting cells (beta-cells) from pancreatic acinar cells has been reported by several groups (18-20). Since a large number of pancreatic exocrine cells can be obtained as a byproduct of islet transplantation, the exocrine pancreas is an intriguing source for the generation of transplantable surrogate beta-cells. Song et al. found that adult rat pancreatic acinar cells transdifferentiated into insulin-expressing cells in vitro (18). They

isolated pancreatic acinar cells from adult rats and cultured the cells in suspension without adding any growth factor and cytokines. Most of the acinar-derived cells lost amylase expression and acquired a ductal phenotype within a week. Insulin-positive cells were detected at the peripheral of the spherical cell clusters derived from the acinar cells (18). Baeyens et al. also have shown that rat exocrine pancreatic cells can transdifferentiate into insulin-secreting cells by cultivation in the presence of EGF and LIF (19). However, neither direct evidence of the origin of these cells nor their precise insulin secretory properties was shown in these studies.

We have established a method for the generation of insulin-secreting cells from pancreatic acinar cells in mice (20). The pancreatic exocrine cell-enriched fraction was prepared by Ficoll density gradient centrifugation. By this method, pancreatic exocrine cells are recovered as a pellet, and are then stained with dithizone, a zinc-chelating agent, to remove contaminated pre-existing pancreatic beta-cells. The resulting fraction contains >90% of amylase-positive cells, approximately 5% of cytokeratin-positive cells, and less than 0.01% of insulin-positive cells. The exocrine cell-enriched fractions were then cultured in RPMI-1640 medium supplemented with 0.5% fetal calf serum (FCS) and 20 ng/ml of EGF. Under these conditions, the cells readily formed aggregates and became smooth spheroids within a few days. When sticky cell culture dishes were used, the cells began to adhere, and formed small monolayer colonies. We found that a subset of the cells in these colonies expressed insulin. Most insulin-positive cells were detected at the peripheral of small colonies. However, insulin secretion was undetectable under these conditions. When the isolated pancreatic exocrine cells were cultured in suspension using dishes treated with 2-methacryloyloxyethyl phosphorylcholine (MPC), which interferes with cell attachment, insulin production was increased compared to that in monolayer culture. Pancreatic exocrine-derived spheroids could be maintained throughout the culture (Figure 2). The frequency of insulin-positive cells was increased to ~5% of total cells on day 4. The insulin-positive cells also expressed C-peptide (Figure 3a), indicating *de novo* biosynthesis of insulin in these cells. To determine the cell type that differentiates into insulin-producing cells in exocrine pancreas, we first evaluated the expression of pancreatic cell markers during culture. Both amylase and elastase, which are acinar cell-specific enzymes, were strongly detected before culture (day 0) at both mRNA (RT-PCR) and protein (immunoblotting) levels. However, their expressions were drastically decreased during culture, becoming barely detectable on day 4 and after. In contrast, the ductal marker CK19 was increased. Immunocytochemistry showed that both amylase- and elastase-positive cells comprised more than 90% of total cells in the initial preparation (day 0), but less than 5% 4 days after culture. While CK-positive cells were rare at day 0, CK-positive ductal structures were found frequently at day 4 and day 6. Although insulin-positive cells were generally negative for amylase, a few cells clearly expressed both insulin and amylase. Amylase/CK double-positive cells also were detected. Similar results were obtained for elastase. Insulin/CK double-positive cells were rarely found, and such cells were not located in well-organized ductal structures (Figure 3b). These results suggest that the insulin-producing cells in the culture are derived from amylase/elastase-expressing mature pancreatic acinar cells.

To confirm that the newly-made insulin-producing cells were derived from pancreatic acinar cells, we utilized the method of cell lineage tracing. In this method, ROSA26-loxP-stop-loxP-ECFP reporter mouse (R26R-ECFP) expressing enhanced cyan fluorescent protein (ECFP) that can be activated through the action of Cre recombinase (96) and adenoviruses in which either amylase-2 (Ad-pAmy-Cre) or elastase-1 (Ad-pEla-Cre) promoter drives the expression of Cre recombinase are used (Figure 4a). Pancreatic exocrine cells from R26R-ECFP mice were infected with these

adenoviruses to label pancreatic acinar cells, and cultured as described.   After the culture, ECFP-expressing insulin-positive cells were frequently found, demonstrating that the insulin-positive cells originate from amylase-expressing pancreatic acinar cells (Figure 4b) (20).

The most important function of pancreatic beta-cells is glucose-induced insulin secretion, in which several key molecules are known to be involved (97).   Glucose transporters, glucokinase, ATP-sensitive K^+ (K_{ATP}) channels, voltage-dependent Ca^{2+} channels, molecules associated with the exocytotic machinery (SNAREs), and prohormone convertases (PC1/3 and PC2) are required for proper glucose-induced insulin secretion.   The expressions of all of these molecules were induced or up-regulated in pancreatic acinar cells after the culture.   The expression profiles of the exocrine pancreas-derived insulin-producing cells became similar to those of the pancreatic islets.   In addition, formation of insulin-containing granules was confirmed by immunoelectron microscopy, indicating that these cells can secrete insulin in a regulated manner.   Indeed, a high concentration of KCl stimulated insulin secretion from the cells, indicating the occurrence of Ca^{2+}-triggered exocytosis in pancreatic acinar-derived cells.   Insulin secretion also was increased by glibenclamide, a sulfonylurea widely used in treatment of diabetes, indicating that functional K_{ATP} channels are expressed.   Importantly, glucose stimulated insulin secretion from exocrine pancreas-derived cells in a concentration-dependent manner, demonstrating that the cells are glucose responsive.   In addition, GLP-1 (7-36 amide), an incretin, potentiated insulin secretion in the presence of relatively high concentrations of glucose, indicating that the cAMP-mediated potentiation system also is present in the cells (Figure 5).   These results show that pancreatic acinar-derived cells have qualitatively similar insulin secretory properties to those of native pancreatic beta-cells. However, insulin content in these pancreatic acinar-derived cells (including both insulin-positive and insulin-negative cells) is only 1/400 that of native pancreatic islets.   Since the frequency of insulin-positive cells is approximately 5% of total cells in the culture, the insulin content of the newly made insulin-positive cells is about 1/20 that of a native beta-cell (20).

6.2. Dedifferentiation during the course of transdifferentiation

During development of the pancreas, several transcription factors are known to play critical roles in beta-cell differentiation (20).   Pdx1, a transcription factor seen early in the developing pancreas and restricted to beta-cells in adults, was induced in pancreatic acinar-derived cells by the culture at both mRNA and protein levels.   Other transcription factors characteristic of endocrine pancreas (NeuroD1, Isl1, Pax6, Nkx2.2, Nkx6.1, Hlxb9, HNF1α, and Foxa1) also were induced.   Foxa2 is expressed in early endoderm (98) and all pancreatic cell types including endocrine and exocrine cells in adult (99).   Although we could not detect Foxa2 expression in isolated acinar cells, probably due to the low expression level, Foxa2 was induced at both mRNA and protein levels after the culture.   HNF6, which is required for the expression of Foxa2 (100), also was induced.   Ptf1a expression was increased by the culture. Although Ptf1a was thought to be an exocrine specific transcription factor (43), a recent study has shown that this factor also is expressed in the pancreatic bud and is required for differentiation into all three cell types (endocrine, exocrine, and duct) of the pancreas (44).   These results suggest that pancreatic acinar cells dedifferentiate into cells having the properties of immature pancreatic cells.   Induction of PGP9.5, a potential marker of endocrine progenitors (101), supports this notion.   Thus, it is likely that isolated pancreatic acinar cells first dedifferentiate into cells with properties of undifferentiated pancreatic cells, and then redifferentiate into insulin-secreting cells under these conditions (Figure 6).

6.3. Signaling mechanisms

We found that enzymatic dissociation of the pancreas leads to activation of the EGF receptor and its downstream signaling. The EGF receptor was not activated before dissociation but was activated (tyrosine phosphrylated) by enzymatic dissociation (Figure 7 and b). When an inhibitor of EGF receptor kinase (AG1478) was applied, transdifferentiation of isolated pancreatic acinar cells into insulin-secreting cells was inhibited (Figure 7c) (20), demonstrating that activation of the EGF receptor is essential for the transdifferentiation. In embryonic pancreas, EGF increases the number of undifferentiated endocrine precursor cells, and, upon removal of EGF, a large number of beta-cells are differentiated (35), suggesting that EGF may be important for the proliferation of endocrine precursors and/or endow the cells with commitment to endocrine lineage. In addition, in TGF-alpha transgenic mice, ductal hyperplasia and pronounced interstitial fibrosis occur in exocrine pancreas (102). In these mice, numerous duct cells of the pancreas have both zymogen and mucin granules (102). Islet neogenesis from the metaplastic duct is observed in the pancreas of these transgenic mice (53). TGF-alpha is a member of the EGF family acting via the EGF receptor. Thus, EGF signaling is also involved in acinar-to-ductal transdifferentiation and islet neogenesis in adult pancreas. In addition, we have found that activation of the PI3-kinase/Akt pathway is essential for *in vitro* transdifferentiation of pancreatic acinar cells into insulin-secreting cells (KM and SS, unpublished observation) (Figure 6).

7. PERSPECTIVE

Generation of pancreatic insulin-secreting cells from non-beta-cells *in vitro* represents a potentially useful approach to cell replacement therapy for type 1 diabetes. Accumulating evidence shows that insulin-secreting cells can be generated from pancreatic acinar cells by transdifferentiation *in vitro* under certain culture conditions. However, such newly made cells are not fully differentiated beta-cells, as assessed by both insulin secretory properties and gene expression profile, compared with native pancreatic beta-cells. Native pancreatic beta-cells are highly differentiated cells equipped with a well-regulated secretory apparatus of insulin secretion that controls blood glucose levels within a narrow physiological range. Considering that the native pancreatic beta-cells form the three-dimensional structure (islet) with other types of endocrine cells, intercellular communication might be an important factor for full differentiation into mature beta-cells from non-beta-cells. In addition, cell-to-extracellular matrix contact could also be implicated in the establishment of cell functions. Therefore, molecules associated with cell-to-cell and cell-to-matrix interactions may have roles in the transdifferentiation. In any case, we need to pay more attention to the morphological aspect of newly-made insulin-secreting cells for the use of cell transplantation. Clarification of the molecular mechanisms of such transdifferentiation as well as the acquisition of insulin secretory function in the process may provide a basis for cell replacement therapy in type 1 diabetes.

8. ACKNOWLEDGMENTS

9. REFERENCES

1. Bonner-Weir, S., and Sharma, A. : Pancreatic stem cells. *J Pathol* 197, 519-26 (2002)

2. Weir, G. C. : Can we make surrogate beta-cells better than the original? *Semin Cell Dev Biol* 15, 347-57 (2004)

3. Halban, P. A. : Cellular sources of new pancreatic beta cells and therapeutic implications for regenerative medicine. *Nat Cell Biol* 6, 1021-5 (2004)

4. Soria, B., Roche, E., Berná, G., León-Quinto, T., Reig, J. A., and Martín, F. : Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes* 49, 157-62 (2000)

5. Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R., and McKay, R. : Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 292, 1389-94 (2001)

6. Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K. L., and Tzukerman, M. : Insulin production by human embryonic stem cells. *Diabetes* 50, 1691-7 (2001)

7. D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., Moorman, M. A., Kroon, E., Carpenter, M. K. , and Baetge, E. E. ：Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 24, 1392-401 (2006)

8. Odorico, J. S., Kaufman, D. S., and Thomson, J. A. : Multilineage differentiation from human embryonic stem cell lines. *Stem Cells* 19, 193-204 (2001)

9. McLaren, A. : Ethical and social considerations of stem cell research. *Nature* 414, 129-31 (2001)

10. Rajagopal, J., Anderson, W.J., Kume, S., Martinez, O.I., and Melton, D.A. : Insulin staining of ES cell progeny from insulin uptake. *Science* 299, 363 (2003)

11. Hansson, M., Tonning, A., Frandsen, U., Petri, A., Rajagopal, J., Englund, M. C., Heller, R. S., Håkansson, J., Fleckner, J., Sköld, H. N., Melton, D., Semb, H., and Serup, P. : Artifactual insulin release from differentiated embryonic stem cells. *Diabetes* 53, 2603-9 (2004)

12. Ramiya, V. K., Maraist, M., Arfors, K. E., Schatz, D. A., Peck, A. B., and Cornelius, J. G. : Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* 6, 278-82 (2000)

13. Bonner-Weir, S., Taneja, M., Weir, G. C., Tatarkiewicz, K., Song, K. H., Sharma, A., and O'Neil, J. J. : In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA* 97, 7999-8004 (2000)

14. Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J. G., Petersen, B. E., and Peck, A. B. : In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci USA* 99, 8078-83 (2002)

15. Gao, R., Ustinov, J., Pulkkinen, M. A., Lundin, K., Korsgren, O., and Otonkoski, T. : Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. *Diabetes* 52, 2007-15 (2003)

16. Tang, D. Q., Cao, L. Z., Burkhardt, B. R., Xia, C. Q., Litherland, S. A., Atkinson, M. A., and Yang, L. J. : In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes* 53, 1721-32 (2004)

17. Slack, J. M. : Developmental biology of the pancreas. *Development* 121, 1569-80 (1995)

18. Song, K. H., Ko, S. H., Ahn, Y. B., Yoo, S. J., Chin, H. M., Kaneto, H., Yoon, K. H., Cha, B. Y., Lee, K. W., and Son, H. Y. : In vitro transdifferentiation of adult pancreatic acinar cells into insulin-expressing cells. *Biochem Biophys Res Commun* 316, 1094-100 (2004)

19. Baeyens, L., De Breuck, S., Lardon, J., Mfopou, J. K., Rooman, I., and Bouwens, L. : In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia* 48, 49-57 (2005)

20. Minami, K., Okuno, M., Miyawaki, K., Okumachi, A., Ishizaki, K., Oyama, K., Kawaguchi, M., Ishizuka, N., Iwanaga, T., and Seino, S. : Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A* 102, 15116-21 (2005)

21. Jørgensen, M. C. , Ahnfelt-Rønne, J., Hald, J., Madsen, O. D., Serup, P., and Hecksher-Sørensen, J. : An illustrated review of early pancreas development in the mouse. *Endocr Rev* 28, 685-705 (2007)

22. Edlund, H. : Pancreas: how to get there from the gut? *Curr Opin Cell Biol* 11, 663-8 (1999)

23. Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. : Notch signaling: cell fate control and signal integration in development. *Science* 284, 770-6 (1999)

24. Hald, J., Hjorth, J. P., German, M. S., Madsen, O. D., Serup, P., and Jensen, J. : Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development. *Dev Biol* 260, 426-37 (2003)

25. Jensen, J. : Gene regulatory factors in pancreatic development. *Dev Dyn* 229, 176-200 (2004)

26. Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabe de Angelis, M., Lendahl, U., and Edlund, H. : Notch signalling controls pancreatic cell differentiation. *Nature* 400, 877-81 (1999)

27. Jensen, J., Heller, R. S., Funder-Nielsen, T., Pedersen, E. E., Lindsell, C., Weinmaster, G., Madsen, O. D., and Serup, P. : Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. *Diabetes* 49, 163-76 (2000)

28. Murtaugh, L. C., Stanger, B. Z., Kwan, K. M., and Melton, D. A. : Notch signaling controls multiple steps of pancreatic differentiation. *Proc Natl Acad Sci U S A* 100, 14920-5 (2003)

29. Bhushan, A., Itoh, N., Kato, S., Thiery, J. P., Czernichow, P., Bellusci, S., and Scharfmann, R. : Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* 128, 5109-17 (2001)

30. Hart, A., Papadopoulou, S., and Edlund, H. : Fgf10 maintains notch activation, stimulates proliferation, and blocks differentiation of pancreatic epithelial cells. *Dev Dyn* 228, 185-93 (2003)

31. Norgaard, G. A., Jensen, J. N., and Jensen, J. : FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development. *Dev Biol* 264, 323-38 (2003)

32. Miralles, F., Lamotte, L., Couton, D., and Joshi, R. L. : Interplay between FGF10 and Notch signalling is required for the self-renewal of pancreatic progenitors. *Int J Dev Biol* 50, 17-26 (2006)

33. Kritzik, M. R., Krahl, T., Good, A., Gu, D., Lai, C., Fox, H., and Sarvetnick, N. : Expression of ErbB receptors during pancreatic islet development and regrowth. *J Endocrinol* 165, 67-77 (2000)

34. Erickson, S. L., O'Shea, K. S., Ghaboosi, N., Loverro, L., Frantz, G., Bauer, M., Lu, L. H., and Moore, M. W. : ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. *Development* 124, 4999-5011 (1997)

35. Cras-Méneur, C., Elghazi, L., Czernichow, P., and Scharfmann, R. : Epidermal growth factor increases undifferentiated pancreatic embryonic cells in vitro: a balance between proliferation and differentiation. *Diabetes* 50, 1571-9 (2001)

36. Böttinger, E. P., Jakubczak, J. L., Roberts, I. S., Mumy, M., Hemmati, P., Bagnall, K., Merlino, G., and Wakefield, L. M. : Expression of a dominant-negative mutant TGF-beta type II receptor in transgenic mice reveals essential roles for TGF-beta in regulation of growth and differentiation in the exocrine pancreas. *EMBO J* 16, 2621-33 (1997)

37. Yamaoka, T., Idehara, C., Yano, M., Matsushita, T., Yamada, T., Ii, S., Moritani, M., Hata, J., Sugino, H., Noji, S., and Itakura, M. : Hypoplasia of pancreatic islets in transgenic mice expressing activin receptor mutants. *J Clin Invest* 102, 294-301 (1998)

38. Vukicevic, S., Helder, M. N., and Luyten, F. P. : Developing human lung and kidney are major sites for synthesis of bone morphogenetic protein-3 (osteogenin). *J Histochem Cytochem* 42, 869-75 (1994)

39. Lyons, K. M., Hogan, B. L., and Robertson, E. J. : Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. *Mech Dev* 50, 71-83 (1995)

40. Jiang, F. X., Stanley, E. G., Gonez, L. J. , and Harrison, L. C. : Bone morphogenetic proteins promote development of fetal pancreas epithelial colonies containing insulin-positive cells. *J Cell Sci* 115, 753-60 (2002)

41. Guz, Y., Montminy, M. R., Stein, R., Leonard, J., Gamer, L. W., Wright, C. V., and Teitelman, G. : Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* 121, 11-8 (1995)

42. Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L., and Wright, C. V. : PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 122, 983-95 (1996)

43. Krapp, A., Knöfler, M., Ledermann, B., Bürki, K., Berney, C., Zoerkler, N., Hagenbüchle, O., and Wellauer, P.K. : The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev* 12, 3752-63 (1998)

44. Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J., and Wright, C. V. : The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat Genet* 32, 128-34 (2002)

45. Melloul, D., Marshak, S., and Cerasi, E. : Regulation of pdx-1 gene expression. *Diabetes* 51, S320-5 (2002)

46. Gradwohl, G., Dierich, A., LeMeur, M., and Guillemot, F. : neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A* 97, 1607-11 (2000)

47. Gu, G., Dubauskaite, J., and Melton, D. A. : Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 129, 2447-57 (2002)

48. Ishizuka, N., Minami, K., Okumachi, A., Okuno, M., and Seino, S. : Induction by NeuroD of the components required for regulated exocytosis. *Biochem Biophys Res Commun* 354, 271-7 (2007)

49. Collombat, P., Mansouri, A., Hecksher-Sorensen, J., Serup, P., Krull, J., Gradwohl, G., and Gruss, P. : Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev* 17, 2591-603 (2003)

50. Huang, X., Cho, S., and Spangrude, G. J. : Hematopoietic stem cells: generation and self-renewal. *Cell Death and Differentiation* 14, 1851-9 (2007)

51. Hawley, R. G., Ramezani, A., and Hawley, T. S. : Hematopoietic stem cells. *Methods Enzymol* 419, 149-79 (2006)

52. Crosnier, C., Stamataki, D., and Lewis, J. : Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* 7, 349-59 (2006)

53. Gu, D., and Sarvetnick, N. : Epithelial cell proliferation and islet neogenesis in IFN-g transgenic mice. *Development* 118, 33-46 (1993)

54. Gu, D., Lee, M. S., Krahl, T., and Sarvetnick, N. : Transitional cells in the regenerating pancreas. *Development* 120, 1873-81 (1994)

55. Gu, D., Arnush, M., and Sarvetnick, N. : Endocrine/exocrine intermediate cells in streptozotocin-treated Ins-IFN-gamma   transgenic mice. *Pancreas* 15, 246-50 (1997)

56. Song, S. Y., Gannon, M., Washington, M. K., Scoggins, C. R., Meszoely, I. M., Goldenring, J. R., Marino, C. R., Sandgren, E. P., Coffey, R. J. Jr., Wright, C. V., and Leach, S. D. : Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* 117, 1416-26 (1999)

57. Miyamoto, Y., Maitra, A., Ghosh, B., Zechner, U., Argani, P., Iacobuzio-Donahue, C. A., Sriuranpong, V., Iso, T., Meszoely, I. M., Wolfe, M. S., Hruban, R. H., Ball, D. W., Schmid, R. M., and Leach, S. D. : Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* 3, 565-76 (2003)

58. Means, A. L., Meszoely, I. M., Suzuki, K., Miyamoto, Y., Rustgi, A. K., Coffey, R. J. Jr., Wright, C. V., Stoffers, D. A., and Leach, S. D. : Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. *Development* 132, 3767-76 (2005)

59. Wang, R. N., Klöppel, G., and Bouwens, L. : Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* 38, 1405-11 (1995)

60. Lardon, J., Huyens, N., Rooman, I., and Bouwens, L. : Exocrine cell transdifferentiation in dexamethasone-treated rat pancreas. *Virchows Arch* 444, 61-5 (2004)

61. Fernandes, A., King, L. C., Guz, Y., Stein, R., Wright, C. V., and Teitelman, G. : Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 138, 1750-62 (1997)

62. Guz, Y., Nasir, I., and Teitelman, G. : Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. *Endocrinology* 142, 4956-68 (2001)

63. Zulewski, H., Abraham, E. J., Gerlach, M. J., Daniel, P. B., Moritz, W., Müller, B., Vallejo, M., Thomas, M. K., and Habener, J. F. : Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 50, 521-33 (2001)

64. Abraham, E. J., Leech, C. A., Lin, J. C., Zulewski, H., and Habener, J. F. : Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* 143, 3152-61 (2002)

65. Bonner-Weir, S., and Sharma, A. : Pancreatic stem cells. *J Pathol* 197, 519-26 (2002)

66. Huang, H., and Tang, X. : Phenotypic determination and characterization of nestin-positive precursors derived from human fetal pancreas. *Lab Invest* 83, 539-47 (2003)

67. Seaberg, R. M., Smukler, S. R., Kieffer, T. J., Enikolopov, G., Asghar, Z., Wheeler, M. B., Korbutt, G., and van der Kooy, D. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol* 22, 1115-24 (2004)

68. Suzuki, A., Nakauchi, H., and Taniguchi, H. : Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. *Diabetes* 53, 2143-52 (2004)

69. Stanger, B. Z., Tanaka, A. J., and Melton, D.A. : Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* 445, 886-91 (2007)

70. Bock, T., Kyhnel, A., Pakkenberg, B., and Buschard, K. : The postnatal growth of the beta-cell mass in pigs. *J Endocrinol* 179, 245-52 (2003)

71. Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A., and Butler, P. C. : Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52, 102-10 (2003)

72. Butler, A. E., Janson, J., Soeller, W. C., and Butler, P. C. : Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes* 52, 2304-14 (2003)

73. Kushner, J. A., Ye, J., Schubert, M., Burks, D. J., Dow, M. A., Flint, C. L., Dutta, S., Wright, C. V., Montminy, M. R., and White, M. F. : Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 109, 1193-201 (2002)

74. Dor, Y., Brown, J., Martinez, O. I., and Melton, D. A. : Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429, 41-6 (2004)

75. Georgia, S., and Bhushan, A. : Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. *J Clin Invest* 114, 963-8 (2004)

76. Teta, M., Rankin, M. M., Long, S. Y., Stein, G. M., Kushner, J.A. : Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell* 12, 817-26 (2007)

77. Brennand, K., Huangfu, D., and Melton, D. : All beta Cells Contribute Equally to Islet Growth and Maintenance. *PLoS Biol* 5, e163 (2007)

78. Shen, C. N., Slack, J. M., and Tosh, D. : Molecular basis of transdifferentiation of pancreas to liver. *Nat Cell Biol* 2, 879-87 (2000)

79. Slack, J. M., and Tosh, D. : Transdifferentiation and metaplasia--switching cell types. *Curr Opin Genet Dev* 11, 581-6 (2001)

80. Tosh, D., and Slack, J. M. : How cells change their phenotype. *Nat Rev Mol Cell Biol* 3, 187-94 (2002)

81. Yeung, R. S., Weese, J. L., Hoffman, J. P., Solin, L. J., Paul, A. R., Engstrom, P. F., Litwin, S., Kowalyshyn, M. J., and Eisenberg, B. L. : Neoadjuvant chemoradiation in pancreatic and duodenal carcinoma. A Phase II Study. *Cancer* 72, 2124-33 (1993)

82. Rao, M. S., Dwivedi, R. S., Subbarao, V., Usman, M. I., Scarpelli, D. G., Nemali, M. R., Yeldandi, A., Thangada, S., Kumar, S., and Reddy, J. K. : Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation. *Biochem Biophys Res Commun* 156, 131-6 (1988)

83. Dabeva, M. D., Hwang, S. G., Vasa, S. R., Hurston, E., Novikoff, P. M., Hixson, D. C., Gupta, S., and Shafritz, D. A. : Differentiation of pancreatic epithelial progenitor cells into hepatocytes following transplantation into rat liver. *Proc Natl Acad Sci U S A* 94, 7356-61 (1997)

84. Reddy, J. K., Rao, M. S., Qureshi, S. A., Reddy, M. K., Scarpelli, D. G., and Lalwani, N. D. : Induction and origin of hepatocytes in rat pancreas. *J Cell Biol* 98, 2082-90 (1984)

85. Miyatsuka, T., Kaneto, H., Shiraiwa, T., Matsuoka, T.A., Yamamoto, K., Kato, K., Nakamura, Y., Akira, S., Takeda, K., Kajimoto, Y., Yamasaki, Y., Sandgren, E.P., Kawaguchi, Y., Wright, C.V., and Fujitani, Y. : Persistent

expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. *Genes Dev* 20, 1435-40 (2006)

86. Strobel, O., Dor, Y., Alsina, J., Stirman, A., Lauwers, G., Trainor, A., Castillo, C. F., Warshaw, A. L., and Thayer, S. P. : In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. *Gastroenterology* 133, 1999-2009 (2007)

87. Okuno, M., Minami, K., Okumachi, A., Miyawaki, K., Yokoi, N., Toyokuni, S., and Seino, S. : Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. *Am J Physiol Endocrinol Metab* 292, E158-65 (2007)

88. Rooman, I., Heremans, Y., Heimberg, H., and Bouwens, L. : Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. *Diabetologia.* 43, 907-14 (2000)

89. Eguchi, G., and Kodama, R. : Transdifferentiation. *Curr Opin Cell Biol* 5, 1023-8 (1993)

90. Echeverri, K., and Tanaka, E. M. : Mechanisms of muscle dedifferentiation during regeneration. *Semin Cell Dev Biol* 13, 353-60 (2002)

91. Yuan, S., Rosenberg, L., Paraskevas, S., Agapitos, D., and Duguid, W. P. : Transdifferentiation of human islets to pancreatic ductal cells in collagen matrix culture. *Differentiation* 61, 67-75 (1996)

92. Schmied, B. M., Ulrich, A., Matsuzaki, H., Ding, X., Ricordi, C., Weide, L., Moyer, M. P., Batra, S. K. , Adrian, T. E., and Pour, P. M. : Transdifferentiation of human islet cells in a long-term culture. *Pancreas* 23, 157-71 (2001)

93. Strobel, O., Dor, Y., Stirman, A., Trainor, A., Fernández-del Castillo, C., Warshaw, A. L., and Thayer, S. P. : Beta cell transdifferentiation does not contribute to preneoplastic/metaplastic ductal lesions of the pancreas by genetic lineage tracing in vivo. *Proc Natl Acad Sci U S A* 104, 4419-24 (2007)

94. Mashima, H., Ohnishi, H., Wakabayashi, K., Mine, T., Miyagawa, J., Hanafusa, T., Seno, M., Yamada, H., and Kojima, I. : Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. *J Clin Invest* 97, 1647-54 (1996)

95. Desai, B. M., Oliver-Krasinski, J., De Leon, D. D., Farzad, C., Hong, N., Leach, S. D., and Stoffers, D. A. : Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J Clin Invest* 117, 971-7 (2007)

96. Srinivas, S., Watanabe, T., Lin, C. S., William, C. M., Tanabe, Y., Jessell, T. M., and Costantini, F. : Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1, 4 (2001)

97. Seino, S. : ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 61, 337-62 (1999)

98. Edlund, H. : Pancreatic organogenesis--developmental mechanisms and implications for therapy. *Nat Rev Genet* 3, 524-32 (2002)

99. Sund, N. J., Vatamaniuk, M. Z., Casey, M., Ang, S. L., Magnuson, M. A., Stoffers, D. A., Matschinsky, F. M., and Kaestner, K. H. : Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev* 15, 1706-15 (2001)

100. Rausa, F., Samadani, U., Ye, H., Lim, L., Fletcher, C. F., Jenkins, N. A., Copeland, N. G., and Costa, R. H. : The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3 beta in the developing murine liver and pancreas. *Dev Biol* 192, 228-46 (1997)

101. Yokoyama-Hayashi, K., Takahashi, T., Kakita, A., and Yamashina, S. : Expression of PGP9.5 in ductal cells of the rat pancreas during development and regeneration: can it be a marker for pancreatic progenitor cells? *Endocr J* 49, 61-74 (2002)

102. Bockman, D.E. and Merlino, G. : Cytological changes in the pancreas of transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* 103, 1883-92 (1992)

FIGURE LEGENDS

Figure 1: The stem cell system. Stem cells in adult tissues are thought to possess capacity for self-renewal and generation of committed progenitor cells. These progenitor cells proliferate and differentiate into several types of functionally differentiated cells.

Figure 2: Pancreatic acinar cell-derived spherical cell clusters. (a) Phase contrast photomicrograph of cultured pancreatic acinar cells. Isolated pancreatic acinar cells were cultured in suspension in the presence of EGF. The cells began to aggregate within a few days and formed smooth spheroids after 4 days of culture. (b) Double immunostaining for F-actin and E-cadherin. F-actin was stained by Alexa Fluor 488-conjugated phalloidin (Molecular Probes; green), and E-cadherin was detected with ECCD-2 anti-E-cadherin antibody (Takara; red). Both F-actin and E-cadherin localized to the plasma membrane region of each cell in the spheroids.

Figure 3: Generation of insulin-positive cells from pancreatic acinar cells. (a) Double immunostaining of insulin and C-peptide. The insulin-positive cells are also positive for C-peptide. Scale bar, 20 μm. (b) Expression of pancreatic cell markers during culture. While amylase-positive cells were markedly decreased, CK-positive ductal structures became apparent by the culture. Insulin/amylase and amylase/CK double-positive cells were detected. Scale bars, 50 μm. Reprinted from Ref. 20 with modification, copyright (2005) National Academy of Science, U.S.A.

Figure 4: Cell lineage tracing by Cre/loxP-based system. (a) The scheme of pancreatic acinar cell specific cell marking. In cells from the R26R-ECFP mouse, expression of the fluorescent protein (ECFP) is activated through the action of Cre recombinase to remove a transcriptional "stop" sequence. When amylase/elastase-expressing acinar cells are infected with adenovirus expressing Cre recombinase under control of either amylase or elastase promoter, the cells are labeled permanently with ECFP. (b) Lineage tracing of labeled acinar cells. Pancreatic acinar cells from R26R-ECFP were labeled by infection of Ad-pAmy-Cre at approximately 50% efficiency. Because fluorescence of ECFP is diminished after fixation, ECFP-expression was detected using anti-GFP antibody. Cells positive for insulin (arrow heads), ECFP (arrows), and both insulin and ECFP (asterisks) are observed. Photographs of higher magnification of insulin/ECFP double-positive cells are shown (lower panels). Scale bars, 20 μm. Reprinted from Ref. 20 with modification, copyright (2005) National Academy of Science, U.S.A.

Figure 5: Insulin secretory properties of pancreatic acinar-derived cells. (a) Immunoelectron microscopic analysis for insulin. Insulin immunoreactivities were detected in the secretory granules. Scale bar, 200 nm. (b) Insulin secretion

in pancreatic acinar-derived cells.   Insulin secretion was stimulated by 30 mM KCl, 0.1 μM glibenclamide (Glib), 0.1 mM carbachol (CCh), or increased concentrations of glucose (G3; 3 mM, G10; 10 mM, G20; 20 mM) for 60 min. Potentiation by GLP-1 (7-36 amide) (100 nM) is also shown. Data are means ± S.E. of three to seven independent experiments.   Reprinted from Ref. 20 with modification, copyright (2005) National Academy of Science, U.S.A.

Figure 6: Model for pancreatic exocrine-to endocrine transdifferentiation.   Enzymatic dissociation of exocrine pancreas disrupts epithelial structures of acini, which causes dedifferentiation of the acinar cells.   Meanwhile, EGF receptors are activated, followed by activation of the PI3-kinase pathway.   Within a few days of culture, spherical cell clusters are formed and genes characteristic of pancreatic beta-cells are induced (redifferentiation).

Figure 7: Involvement of EGF signaling in acinar cell transdifferentiation. (a) Tyrosine phosphorylation of cellular protein. In dissociated pancreatic cells (+), tyrosine phosphorylation was increased compared to undissociated pancreas (-). Anti-phosphotyrosine antibody (PY20) was used for detection. (b) Phosphorylation of EGFR. Tyrosine phosphorylation of the EGFR was detected in dissociated pancreatic cells. (c) Effect of AG1478 on acinar cell transdifferentiation. AG1478 strongly inhibited induction of genes indicating transdifferentiation.   Reprinted from Ref. 20 with modification, copyright (2005) National Academy of Science, U.S.A.

Key words: Transdifferentiation; Pancreatic beta-cells; Pancreatic acinar cells; Regeneration

Send correspondence to: Kohtaro Minami, Ph.D., Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan, Tel: +81-78-304-6046, Fax: +81-78-304-6057, E-mail: kminami@btctr.kobe-u.ac.jp

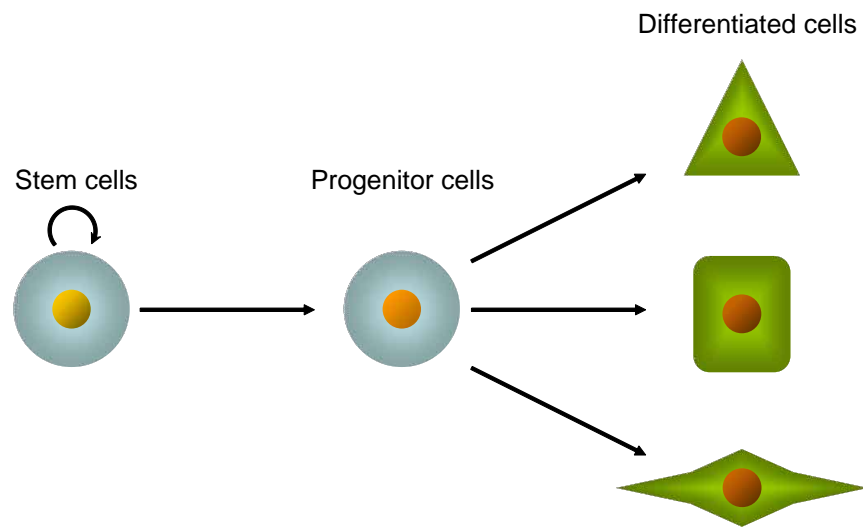**Running title**: Pancreatic acinar-to-endocrine transdifferentiation

Stem cells

Progenitor cells
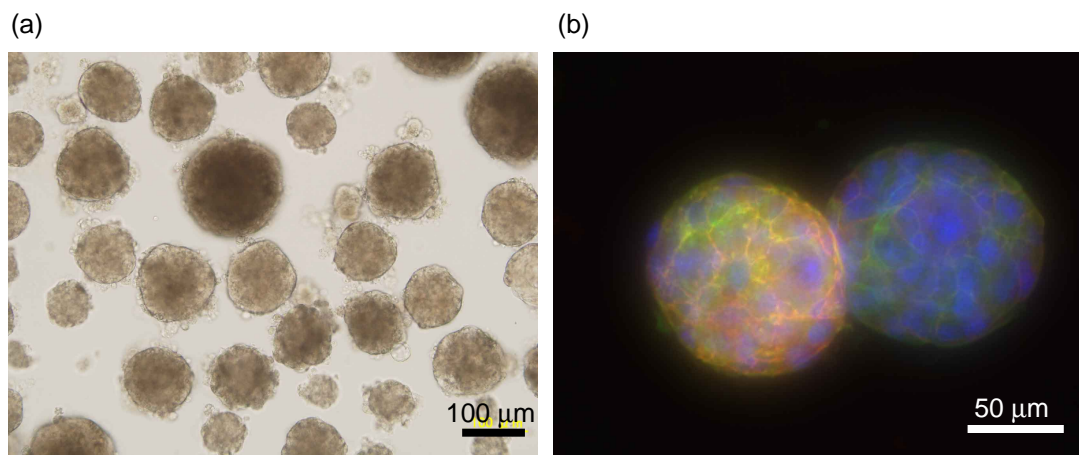
Differentiated cells

Figure 1 (Minami and Seino)

(a)

(b)

100 µm

50 µm

Figure 2 (Minami and Seino)

(a)

Insulin | C-peptide | Merge

(b)

| | Day 0 | Day 2 | Day 4 | | Day 6 |

Insulin/Amylase

Insulin/CK

Amylase/CK
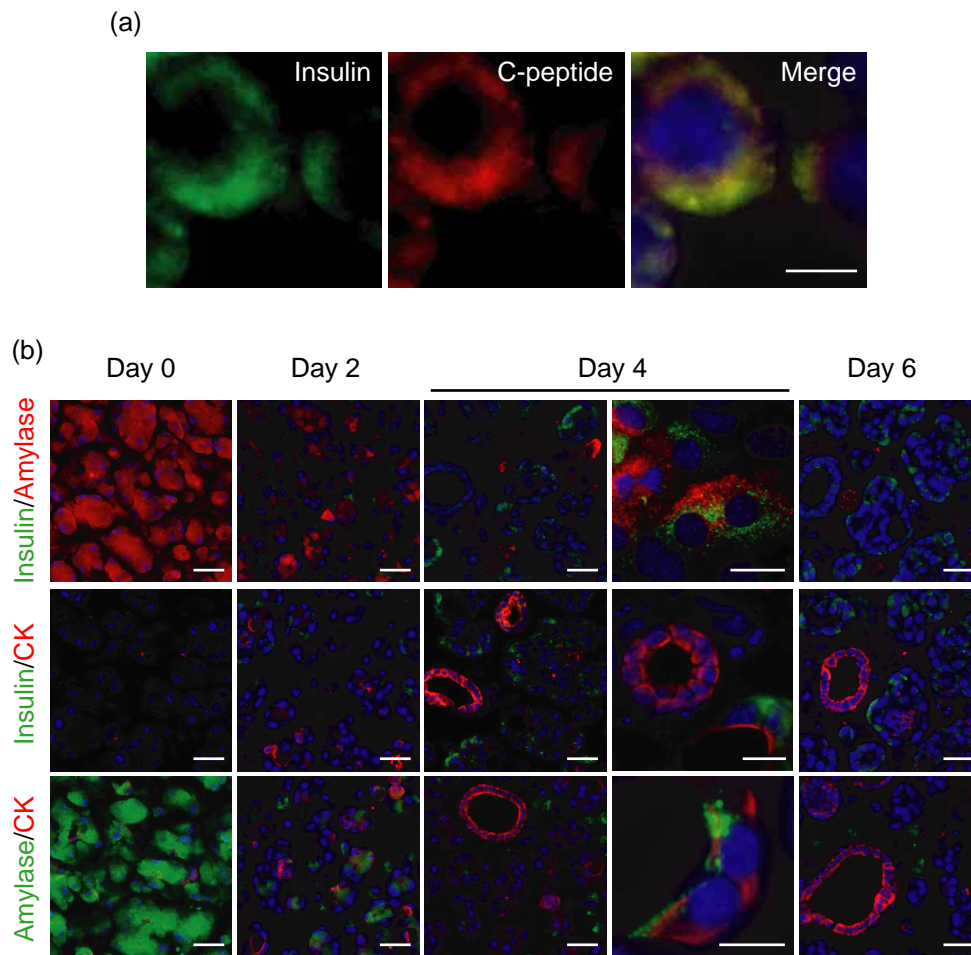
Figure 3 (Minami and Seino)

(a)

Tg mouse — ROSA26 ▮ ▯ ECFP pA —
loxP loxP
Stop

Infection ✕

Adenovirus
— pAmylase Cre pA —
or
— pElastase Cre pA —

Recombination ⬇

— ROSA26 ▮ ECFP pA —
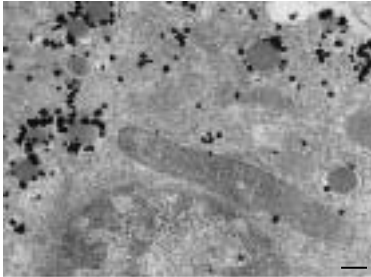→ Expression

(b)

Insulin | ECFP | Merge
Insulin | ECFP | Merge
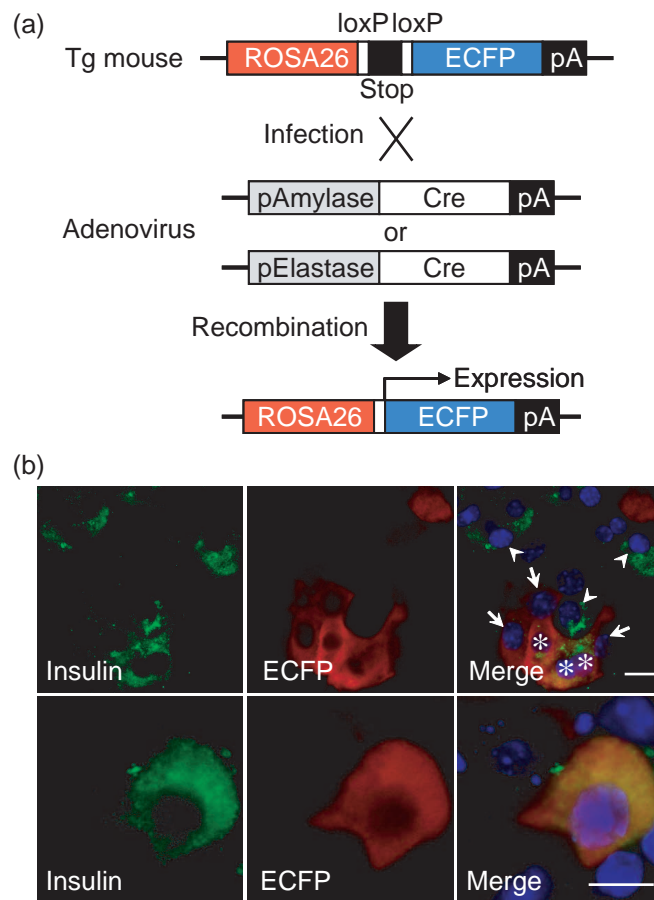
Figure 4 (Minami and Seino)

(a)

(b)
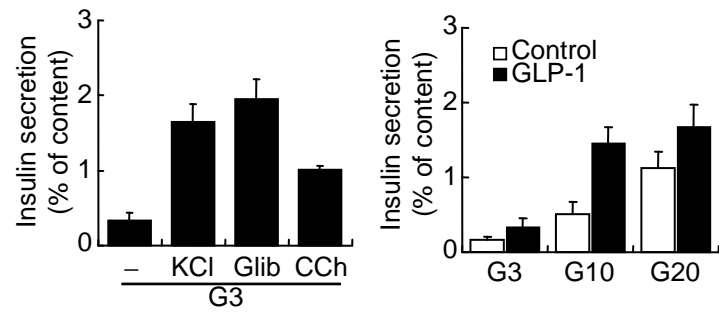
Figure 5 (Minami and Seino)

Isolated acinar cells

Dedifferentiation

Dedifferentiated cells

Redifferentiation
(activation of PI3-kinase)

Insulin-secreting cells

Figure 6 (Minami & Seino)

(a)

Dissociation
(–) (+) (kDa)

– 200

– 116

– 97

– 66

(b)

Dissociation
(–) (+)

pTyr

EGFR

(c)

Day 0
(–)
AG1478
EGF
EGF + AG

18S RNA
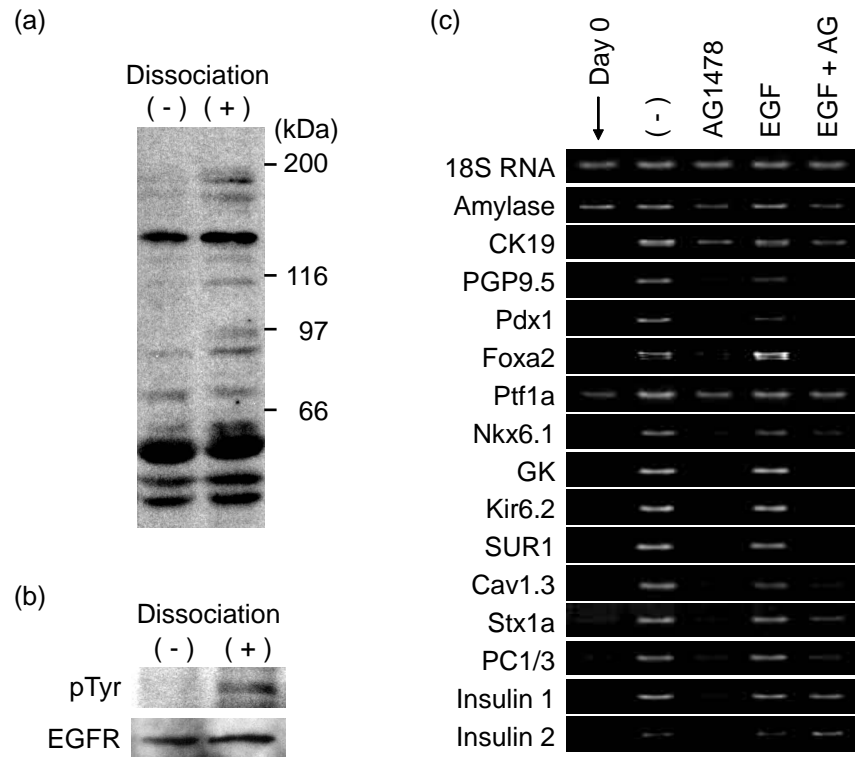Amylase
CK19
PGP9.5
Pdx1
Foxa2
Ptf1a
Nkx6.1
GK
Kir6.2
SUR1
Cav1.3
Stx1a
PC1/3
Insulin 1
Insulin 2
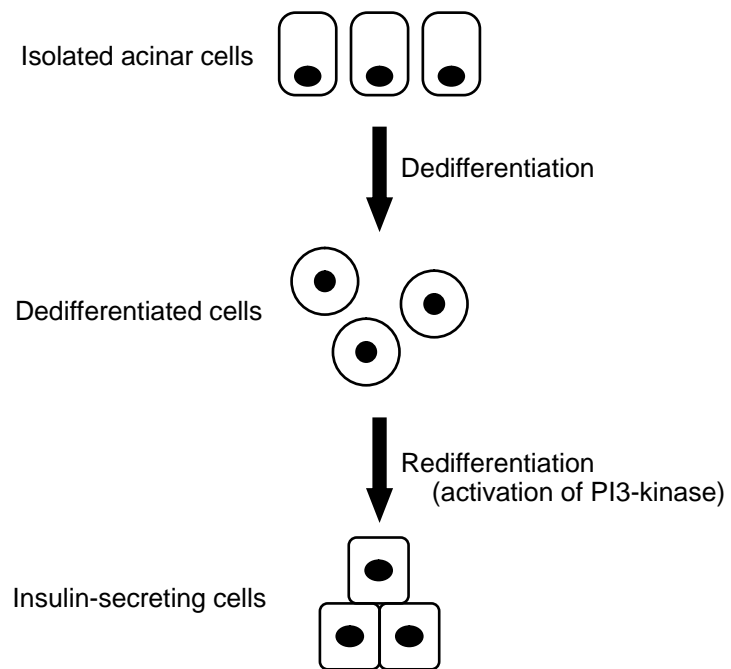
Figure 7 (Minami and Seino)