In vitro generation of insulin-secreting cells from human pancreatic exocrine cells

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ABSTRACT

Transplantation of surrogate β-cells is a promising option for the treatment of insulin-deficient diabetes mellitus in the future. Although pancreatic exocrine cells of rodents have been shown to transdifferentiate into insulin-secreting cells, no studies are reported on human exocrine cells. Here, we report the generation of insulin-secreting cells from exocrine cells of the human pancreas. When cultured in suspension with epidermal growth factor, human pancreatic exocrine cells readily formed spherical cell clusters. Expression of Pdx1 was induced in all 19 cases in which we successfully isolated exocrine cells, and insulin expression was induced in 11 cases. In addition, insulin secretion was evaluated in four cases, and the newly-made cells were found to secrete insulin in response to various stimuli. Although further studies are required to improve both the quality and quantity of such insulin-secreting cells, our data suggest that pancreatic exocrine cells represent a potential source of insulin-secreting cells for treatment of type 1 diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00095.x, 2011)

KEY WORDS: Pancreatic exocrine cells, Insulin secretion, Transdifferentiation

INTRODUCTION

In vitro generation of insulin-secreting cells and transplantation of such surrogate β-cells is a promising option for the treatment of insulin-deficient diabetes mellitus. There are many possible sources of *in vitro*-generated insulin-secreting cells, including pluripotent stem cells and embryonic stem cells[1], pancreatic exocrine cells[2–5], liver cells[6] and pancreatic β-cells themselves[7]. Among these cells, pancreatic exocrine cells are close relatives of β-cells inasmuch as all pancreatic cells have the same developmental origin from a common progenitor that gives rise to both of them[8]. Although it might seem problematic that functionally differentiated cells can change their properties, mature pancreatic exocrine cells are well-known to retain plasticity of their phenotype. Indeed, the present authors and others have shown that pancreatic exocrine (acinar) cells of rodents can transdifferentiate into β-cell-like insulin-secreting cells *in vitro*[2–5]. *In vivo* reprogramming of pancreatic exocrine cells into β-cells has also been reported recently[9]. In addition, an abundant source of pancreatic exocrine cells is readily available as a byproduct of islet transplantation. Thus, pancreatic exocrine cells are a potential source for generation of new β-cells. However, conversion of human pancreatic exocrine cells into insulin-secreting cells has not been reported so far.

In the present study, we obtained human pancreatic tissues from patients who underwent pancreatectomy, and showed that insulin-secreting cells can be generated from human pancreatic exocrine cells.

MATERIALS AND METHODS

Cell Isolation and Culture

Human pancreatic tissues were obtained with informed consent from 32 patients who underwent pancreatectomy at Kyoto University Hospital. Non-malignant parts (0.5–5 g) of the resected specimens were immediately processed for cell isolation. Briefly, the tissues were cut into small pieces and digested by collagenase P (Roche Diagnostics, Basel, Switzerland). The digested tissues were filtrated and the cells were then stained with dithizone, which marks insulin-containing granules, to remove pre-existing pancreatic β-cells by hand-picking[4]. All experiments were approved by the Ethics Committees of Kobe University Graduate School of Medicine and of Kyoto University Graduate School and Faculty of Medicine.

Cell Analyses

For immunostaining, cultured cells were fixed and permeabilized. Guinea pig anti-insulin antibody (Zymed, San Francisco, CA, USA) was used as the primary antibody, and the secondary antibody labeled with Alexa Fluor 488 (Molecular Probe, Eugene, OR, USA) was used for detection. Reverse transcription

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polymerase chain reaction (RT–PCR) analysis and measurement of insulin secretion were carried out as described previously[4,10]. Detailed methods are found in Appendix S1.

RESULTS

Isolation and Culture of Human Pancreatic Exocrine Cells

Human pancreatic tissues were obtained from a total of 32 patients who underwent pancreatectomy (Table 1). However, in 11 cases, cells could not be isolated by collagenase digestion, probably because of severe fibrosis caused by chronic pancreatitis. Most of such cases were patients with pancreatic cancer. Pancreatic cells were successfully isolated in the remaining 21 cases and cultured after removal of pre-existing pancreatic β-cells stained with dithizone[4]. In two cases, however, the cell number was too low for further analysis. The human pancreatic exocrine cells aggregated together immediately after culture and formed spherical cell clusters within 1 day in the presence of 20 ng/mL epidermal growth factor (EGF; Figure 1a). As seen in pancreatic exocrine cells of mice[4], enzymatic dissociation spontaneously activated EGF signaling and the spherical clusters were formed without EGF, but the addition of EGF in the media enhanced and prolonged activation of the signaling.

Transdifferentiation of Human Pancreatic Exocrine Cells to β-Cell-Like Cells

We then determined gene expression of the cultured pancreatic exocrine cells by quantitative real-time RT–PCR. Expression of Pdx1, the master regulator of pancreas development and β-cell function, was induced in all cases analyzed (Table 1). Insulin expression was also induced in 11 cases. In addition, glucokinase and SUR1 were upregulated in 13 and 17 cases, respectively. The average values of gene expression, including other genes associated with pancreatic endocrine cells, are shown in Figure 2. Expressions of exocrine markers, amylase and elastase,

Table 1 | Summary of experiments

Case	Sex	Age (years)	Cell isolation	Gene induction				Insulin secretion
				Insulin	Pdx1	Glucokinase	SUR1	
1 Intraductal papillary mucinous neoplasm	F	58	Fair	N.D.	N.D.	N.D.	N.D.	–
2 Pancreatic cancer	F	60	×	–	–	–	–	–
3 Intraductal papillary mucinous neoplasm	F	66	Good	Yes	Yes	Yes	Yes	N.D.
4 Cholangiocarcinoma	F	60	Fair	No	Yes	No	Yes	–
5 Pancreatic cancer	F	58	Fair	No	Yes	No	Yes	–
6 Intraductal papillary mucinous neoplasm	M	73	Good	Yes	Yes	Yes	Yes	Yes
7 Pancreatic cancer	F	73	Fair	No	Yes	No	Yes	–
8 Intraductal papillary mucinous neoplasm	M	59	Fair	No	Yes	Yes	Yes	–
9 Pancreatic cancer	F	57	×	–	–	–	–	–
10 Pancreatic cancer	M	42	Fair	No	Yes	No	No	–
11 Intraductal papillary mucinous neoplasm	F	67	Good	Yes	Yes	Yes	Yes	N.D.
12 Pancreatic cancer	M	76	×	–	–	–	–	–
13 Duodenal cancer	F	58	Good	Yes	Yes	Yes	Yes	N.D.
14 Pancreatic cancer	M	56	Fair	N.D.	N.D.	N.D.	N.D.	–
15 Intraductal papillary mucinous neoplasm	M	59	Fair	No	Yes	No	No	–
16 Pancreatic cancer	M	56	Fair	No	Yes	Yes	Yes	–
17 Pancreatic cancer	F	68	×	–	–	–	–	–
18 Pancreatic cancer/Stomach cancer	M	56	Good	Yes	Yes	Yes	Yes	N.D.
19 Pancreatic cancer	F	72	×	–	–	–	–	–
20 Intraductal papillary mucinous neoplasm	M	64	Fair	No	Yes	No	Yes	–
21 Pancreatic cancer	F	56	×	–	–	–	–	–
22 Pancreatic cancer	M	74	×	–	–	–	–	–
23 Pancreatic cancer	M	71	×	–	–	–	–	–
24 Cystadenocarcinoma	F	61	Good	Yes	Yes	Yes	Yes	Yes
25 Intraductal papillary mucinous neoplasm	M	59	Good	Yes	Yes	Yes	Yes	N.D.
26 Pancreatic cancer	F	60	×	–	–	–	–	–
27 Pancreatic cancer	M	70	Good	Yes	Yes	Yes	Yes	Yes
28 Intraductal papillary mucinous neoplasm	F	62	Good	Yes	Yes	Yes	Yes	N.D.
29 Pancreatic cancer	M	60	×	–	–	–	–	–
30 Pancreatic cancer	F	66	Good	Yes	Yes	Yes	Yes	N.D.
31 Intraductal papillary mucinous neoplasm	M	53	Good	Yes	Yes	Yes	Yes	Yes
32 Duodenal cancer	M	57	×	–	–	–	–	–

×, cell isolation failed; N.D., not determined because of low cell number.

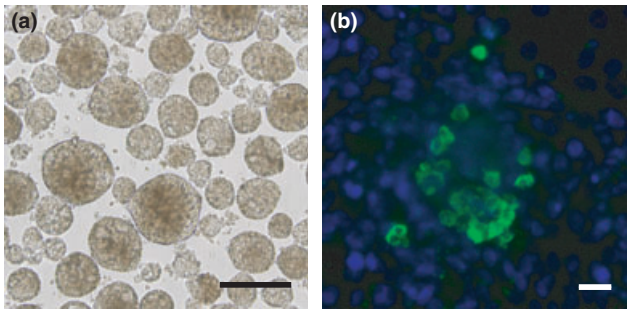

Figure 1 | Culture of isolated human pancreatic exocrine cells. (a) Morphology. When isolated human pancreatic exocrine cells were cultured in suspension in the presence of epidermal growth factor, the cells formed spherical cell clusters with a smooth surface. Bar, 200 μm. (b) Immunostaining. A representative immunofluorescence photomicrograph from case 6 is shown. Insulin-positive cells were detected. Bar, 50 μm.

were lost during culture. These results suggest that human pancreatic exocrine cells change their property to β-cell-like cells by culture with EGF. Indeed, insulin-positive cells were detected by immunostaining (Figure 1b). Expressions of glucagon and somatostatin were also induced (Figure 2). The present data show that pancreatic endocrine cells were generated from human pancreatic exocrine cells, as was found in pancreatic exocrine cells from mice[5].

Insulin Secretion

Insulin secretion was examined in four cases in which both the quality and quantity of the cells obtained were sufficiently high for analysis. Because the amount of secreted insulin varied among these cases, independent results are shown (Figure 3). The newly-made cells derived from human pancreatic exocrine cells secreted insulin in response to glucose, a high concentration of KCl and glibenclamide. In the most successful case (case 24), total insulin content in the insulin-secreting cells was 32.5 U. Because the insulin content of a native human pancreatic islet is approximately 2.4 mU (based on our own measurement), the newly-made cells are equivalent to 13,500 islets. In this case, we used 5 g of starting tissue. Thus, if a whole pancreas (approximately 75 g) was used, an approximately 200,000-islet equivalent could be obtained.

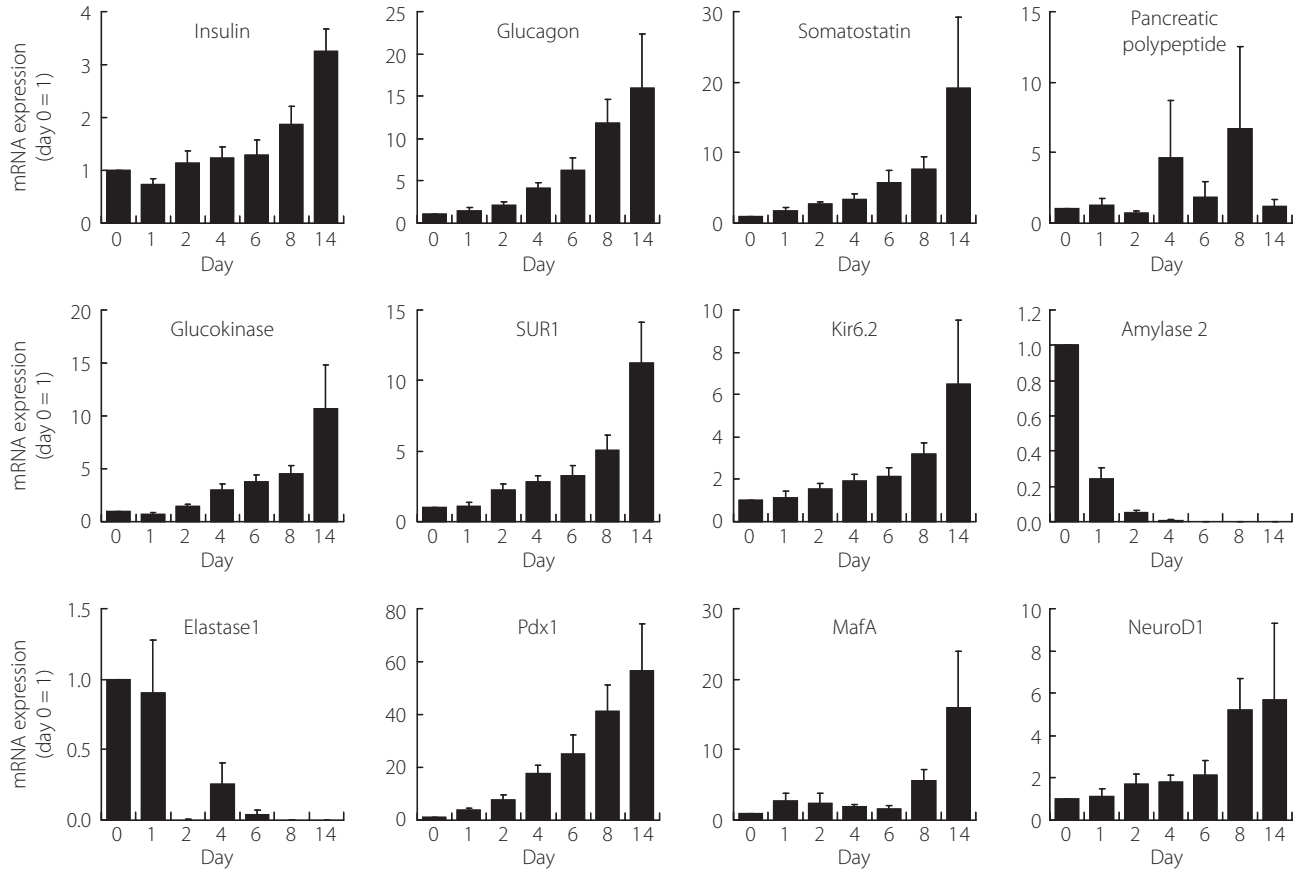

Figure 2 | Changes in gene expression of cultured human pancreatic acinar cells. mRNA expression was determined by quantitative real-time reverse transcription polymerase chain reaction analysis using TaqMan probes. Expression levels of the listed genes were normalized by 18S rRNA expression and are shown relative to those of at day 0. Values are means with SE bars (n = 19).

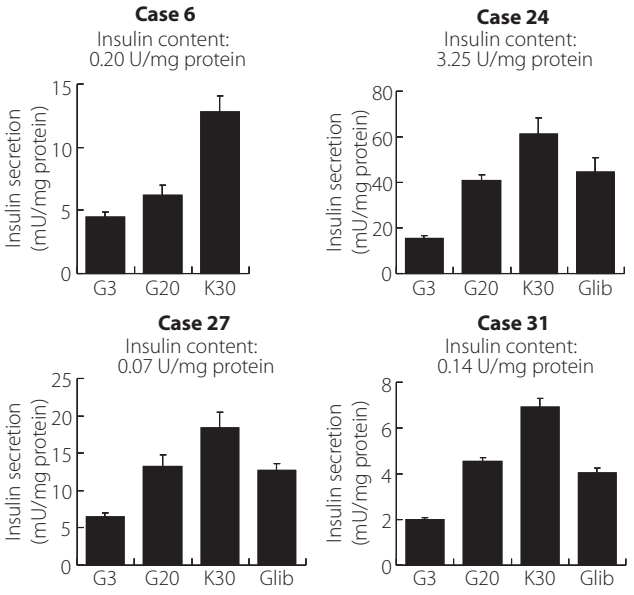

Figure 3 | Insulin secretion from the newly-made cells. Insulin secretion was stimulated by glucose (G3; 3 mmol/L, G20; 20 mmol/L), 30 mmol/L KCl and 0.1 μmol/L glibenclamide (Glib) for 60 min. Secretion was normalized by total cellular protein content. Data are means of four measurements from a single experiment in each case.

DISCUSSION

In the present study, we showed that insulin-secreting cells can be generated from human pancreatic exocrine cells. Because a large number of pancreatic exocrine cells can be obtained as a byproduct of islet transplantation, our finding will further the development of a novel cell-based therapy for diabetes in the future.

It is noteworthy that Pdx1 expression was strongly induced in cultured pancreatic exocrine cells in all cases. Pdx1 is known to be the master regulator of pancreas development and is also a critical transcription factor in the acquisition of β-cell function[11]. In our previous study using mouse pancreatic exocrine acinar cells[4,5,10], induction of Pdx1 expression was also detected under the same culture conditions used in the present study. We have shown that isolated mouse pancreatic acinar cells first dedifferentiate into undifferentiated cells and then redifferentiate into insulin-secreting cells[10,12]. It is therefore likely that human cultured pancreatic exocrine cells dedifferentiate into Pdx1-expressing cells with somewhat immature pancreatic cell properties and that a part of these cells then begin to express insulin and genes specific to pancreatic β-cells.

Although there are considerable similarities between mouse and human pancreatic exocrine cell cultures, they differ in several respects. For example, we noticed that spherical cell clusters with a smooth surface form in a shorter period in human cells than in mouse cells. Furthermore, in contrast to the drastic increase in Pdx1 expression, induction of insulin expression was relatively weak in human cells. Insulin content in the newly-made insulin-secreting cells was only approximately 2% of that in native human islets (7.2 U/mg protein), except for case 24 (see Figure 3). In cases in which insulin-secreting cells could not be generated, the quality of the exocrine cells isolated was low because of severe fibrosis as a result of pancreatitis. In any case, optimization of culture conditions is necessary to improve both the quality and quantity of newly-made insulin-secreting cells derived from human pancreatic exocrine cells.

In general, more than one donor pancreas is required for islet transplantation to achieve insulin-independence in patients with type 1 diabetes[13]. However, the shortage of donor organs for islet transplantation is inevitable. Generation of insulin-secreting cells from pancreatic exocrine cells, which can be obtained as a byproduct of islet isolation, could complement the islet transplantation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 | Materials and Methods.

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