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Culture Conditions for Mouse Pancreatic Stem Cells

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Recently, mouse pancreatic stem cells have been isolated from adult mouse pancreata. However, these pancreatic stem cells could be maintained only under specific culture conditions with lot-limited fetal bovine serum (FBS). For the efficient isolation and maintenance of mouse pancreatic stem cells, it is important to identify culture conditions that can be used independent of the FBS lot. In this study, we evaluated the culture conditions required to maintain mouse pancreatic stem cells. The mouse pancreatic stem cells derived from the pancreas of a newborn mouse, HN#101, were cultured under the following conditions: 1) Dulbecco's modified Eagle's medium (DMEM) with 20% lot-limited FBS, in which mouse pancreatic stem cells could be cultured without changes in morphology and growth activity; 2) complete embryonic stem (ES) cell media; and 3) complete ES cell media on feeder layers of mitomycin C-treated STO cells, which were the same culture conditions used for mouse ES cells. Under culture conditions #1 and #3, the HN#101 cells continued to form a flat "cobblestone" monolayer and continued to divide actively beyond the population doubling level (PDL) 100 without growth inhibition, but this did not occur under culture condition #2. The gene expression profile and differentiated capacity of the HN#101 cells cultured for 2 months under culture condition #3 were similar to those of HN#101 cells at PDL 50. These data suggest that complete ES cell media on feeder layers could be useful for maintaining the undifferentiated state of pancreatic stem cells.

Key words: Mouse pancreatic stem cells; Culture condition; Embryonic stem cells; Embryonic stem (ES) medium; Feeder cells; Pancreatic islet transplantation

INTRODUCTION

β-Cell replacement therapy via islet transplantation represents a promising treatment for type 1 diabetes (6,11,16–18,21). However, such an approach is severely limited by the shortage of donor organs. Pancreatic stem/progenitor cells could be a useful target for β-cell replacement therapy in diabetic patients, since the cells are abundantly available in the pancreas of these patients as well as in donor organs. We recently established mouse pancreatic stem cells without genetic manipulation (19). The clonal cell line obtained (HN#13) expresses the pancreatic and duodenal homeobox factor 1 (Pdx1), also known as islet/duodenal homeobox-1 (IDX-1)/somatostatin transcription factor 1 (STF-1)/insulin promoter factor 1 (IPF1), one of the transcription factors specific for the β-cell lineage. Induction therapy with exendin-4 and with the Pdx1 and β-cell E-box transactivator/neuronal differentiation 1 (BETA2/NeuroD) transcription factors using protein transduction technology (9,10,12–14) stimulated the expression of insulin mRNA in the cells. Yamamoto et al. also reported the generation of mouse pancreatic stem cells using serum-free medium containing cholera toxin, which stimulated cAMP signaling in the cells (24). These pancreatic stem cells have the potential to differentiate into not only insulin-producing cells but also hepatocytes (19,24). These isolation techniques might also be useful for the identification and isolation of human pancreatic stem/progenitor cells.

On the other hand, the HN#13 cells could be maintained in Dulbecco's modified Eagle's medium (DMEM) with only 1 of 10 different lots of fetal bovine serum (FBS) (BIO-

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WEST, Inc., Logan, UT, USA; S1560 Lot. #SO5094S1560). The cells in the other DMEM-FBS formed "fibroblast-like" or "spindle" structures and stopped dividing after three to six passages (19). For efficient isolation and maintenance of mouse pancreatic stem cells, it is important to identify culture conditions that are independent of the FBS lot. In this study, we evaluated the culture conditions required to maintain mouse pancreatic stem cells.

MATERIALS AND METHODS

Isolation and Culture of Mouse Pancreatic Stem Cells and Islets

The mouse studies were approved by the review committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. Islets were isolated from the pancreata of 8-week-old mice (C57BL/6; CLEA Japan, Inc., Meguro, Tokyo, Japan). For islet isolation,the common bile duct was cannulated and injected with 2 ml of cold M199 medium (Life Technologies Japan, Tokyo, Japan) containing 2 mg/ml collagenase (Roche Boehringer Mannheim, Indianapolis, IN, USA) (14). The islets were separated on a density gradient (Optiprep®, Sigma-Aldrich, St. Louis, MO, USA), hand-picked under a dissecting microscope to ensure a pure islet preparation, and used immediately afterward.

Pancreatic stem cells from newborn mice were isolated using a modification of a method that was reported previously (19). In brief, the pancreatic tissue from newborn mice was digested by 2 mg/ml collagenase, and the digested cells were then cultured in DMEM (Life Technologies) with 20% FBS (BIO-WEST, Inc., S1560 Lot. #SO5094S1560). After the cells were attached and spread, nonductal cells (fibroblast morphology) were removed mechanically with a rubberscrapper (Life Technologies). The "duct-like" cells were then inoculated into 96-well plates (Life Technologies), cloned by limiting dilution, and cultured in DMEM with 20% FBS.

Culture Conditions

Mouse pancreatic stem cells from newborn mice were cultured under the following conditions: 1) DMEM with 20% FBS(BIO-WEST, Inc., S1560 Lot. #SO5094S1560), 2) complete embryonic stem (ES) cell media with 15% FBS (Millipore, Billerica, MA, USA), 3) complete ES cell media with 15% FBS on feeder layers of mitomycin C (Sigma-Aldrich)-treated STO cells [Sandos inbred mice fibroblast cell line with 6-thioguanine and ouabain resistance; American Type Culture Collection (ATCC), Manassas, VA, USA] (the same as the culture conditions used for mouse ES cells) (Fig. 1A).

ES Cell Culture and Differentiation

Mouse ES cells (ATCC) were maintained in complete ES cell media with 15% FBS on feeder layers of mitomycin C-treated STO cells. ES cells were passaged every 3 days. The cells that differentiated from ES cells (generated by a stepwise differentiation protocol that relies on intermediates thought to be similar to cell populations present in the developing embryo) were used as a positive control. Directed differentiation was conducted as described previously (2,5), with minor modifications. In stage 1, cells were treated with 25 ng/ml of wingless-type MMTV integration site family, member 3A (Wnt3a) and 100 ng/ml of activin A (R&D Systems, Minneapolis, MN, USA) in Roswell Park Memorial Institute media (RPMI; Life Technologies) for 1 day, followed by treatment with 100 ng/ml of activin A in RPMI+0.2% FBS for 2 days. In stage 2, the cells were treated with 50 ng/ml of fibroblast growth factor 10 (FGF10; R&D Systems) and 0.25 μM of 3-keto-*N*-(aminoethyl-aminocaproyl-dihydrocinnamoyl) cyclopamine (KAAD-cyclopamine; Toronto Research Chemicals, Ontario, Canada) in RPMI+2% FBS for 3 days. In stage 3, the cells were treated with 50 ng/ml of FGF10, 0.25 μM of KAAD-cyclopamine, and 2 μM of all-*trans* retinoic acid (Sigma-Aldrich, Tokyo, Japan) in DMEM+1% (v/v) B27 supplement (Life Technologies) for 3 days. ES cells differentiated into definitive endoderm in stage 1, into gut tube endoderm in stage 2, and then into pancreatic progenitors in stage 3.

Semiquantitative RT-PCR

Total RNA was extracted from cells using an RNeasy Mini Kit (QIAGEN, Tokyo, Japan). After quantifying the RNA by spectrophotometry, 2.5 μg of RNA were heated at 85°C for 3 min and then reverse-transcribed into cDNA in a 25-μl solution containing 200 U of Superscript II RNase H-RT (Life Technologies), 50 ng random hexamers (Life Technologies), 160 μmol/L dNTP, and 10 nmol/L dithiothreitol. The reaction consisted of 10 min at 25°C, 60 min at 42°C, and 10 min at 95°C. Polymerization reactions were performed in a Perkin-Elmer 9700 Thermocycler with 3 μl cDNA (20 ng RNA equivalents), 160 μmol/L cold dNTPs, 10 pmol appropriate oligonucleotide primers, 1.5 mmol/L $MgCl_2$, and 5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, CT, USA). The oligonucleotide primers and cycle numbers used for semiquantitative PCR are shown in Table 1. The thermal cycle profile used a 10-min denaturing step at 94°C, followed by amplification cycles (1 min denaturation at 94°C, 1 min annealing at 57°C, and 1 min extension at 72°C) with a final extension step of 10 min at 72°C. The steps taken to validate these measurements have all been reported previously (12).

Cell Induction and Differentiation Into Insulin-Producing Cells

To induce cellular differentiation, the cells were cultured in DMEM with 10% FBS, 10 nM exendin-4, 10 mM nicotinamide, 10 ng/ml heratinocyte growth factor

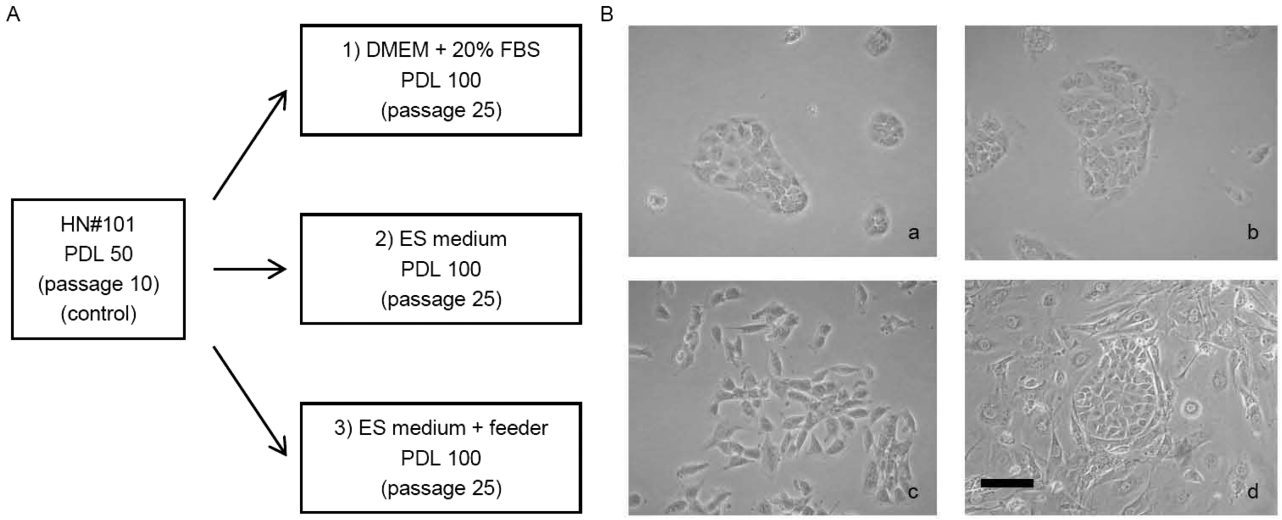

Figure 1. Isolation and culture of pancreatic stem cells. (A) Study design. HN#101 cells (PDL 50) were cultured under the following conditions: 1) DMEM with 20% FBS, 2) complete ES cell media with 15% FBS, 3) complete ES cell media with 15% FBS on feeder layers of mitomycin C-treated STO cells (the same as the culture conditions used for mouse ES cells). (B) The morphology of the pancreatic stem cells isolated from a newborn mouse, HN#101 (PDL 50). After digestion of pancreatic tissue, the "duct-like" cells were inoculated into 96-well plates, cloned by limiting dilution, and cultured in DMEM with 20% FBS. (a) The morphology of the HN#101 cells in DMEM with 20% FBS (PDL 50). (b) The morphology of the HN#101 cells in DMEM with 20% FBS (PDL 100). (c) The morphology of the HN#101 cells in complete ES cell media with 15% FBS (PDL 100). (d) The morphology of the HN#101 cells in complete ES cell media with 15% FBS on feeder layers of mitomycin C-treated STO cells (Sandos inbred mice fibroblast cell line with 6-thioguanine and ouabain resistance; PDL 100). Scale bar: 100 μm.

(KGF; all Sigma-Aldrich), 100 nM Pdx1 protein, and 100 nM BETA2/NeuroD protein for 7 to 10 days. For the Pdx1 and BETA2/NeuroD proteins, the cDNAs were amplified by PCR using appropriate linker primers and then were subcloned into the *Nde* I and *Xho* I sites of pET21b(+) (Novagen, Madison, WI, USA) using a ligation kit (TaKaRa, Tokyo, Japan). BL21 (DE3) cells (Life Technologies) containing the expression plasmids were grown at 37°C to an OD_{600} of 0.8. Next, isopropyl-β-D-thiogalactopyranoside (Sigma-Aldrich) was added to a final concentration of 0.1 mmol/L, and the cells were then incubated for 12 h at 24°C. Cells were sonicated, and the supernatants were recovered and applied to a column of Ni-nitrilotriacetic acid agarose (Life Technologies).

Table 1. List of Gene-Specific Primers

Gene	Forward/Reverse Primer
Sox17	ctgccctgccgggatggcacggaatc/ ttctggccctcaggtcgggtcggcaac
Foxa2	tggtcactggggacaagggaa/gcaacaacagcaatagagaac
Hnf1β	cacagccctcaccagcagcc/gactgcctgggctctgctgc
Hnf4α	acacgtccccatctgaaggtg/cttccttcttcatgccagccc
Pdx1	cctgcgtgcctgtacatggg/tttccacgcgtgagctttgg
Hnf6	gggtgagccatgagccggtg/catagccgcgccgggatgag
Insulin-1	ccagctataatcagagacca/gtgtagaagaagccacgct
Insulin-2	tccgctacaatcaaaaaccat/gctgggtagtggtgggtcta
Glucagon	actcacagggcacattcacc/ccagttgatgaagtccctgg
NeuroD	gcgctcaggcaaaagccc/gccattgatgctgagcggcg
Isl-1	agatatgggagacatgggcgat/acacagcggaaacactcgatg
Pax6	cagtcacagcggagtgaatc/cgcttcagctgaagtcgcat
GAPDH	accacagtccatgccatcac/tccaccaccctgttgctgta

Sox 17, sex-determining region Y-box17; Foxa2, forkhead box protein a2; Hnf, hepatocyte nuclear factor; Pdx1, pancreatic and duodenal homeobox factor-1; NeuroD, neuronal differentiation 1; Isl-1, islet or insulin gene enhancer protein; Pax6, paired box gene 6; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Isolation of Pancreatic Stem Cells

Pancreatic stem cells were isolated from the pancreata of newborn mice. After digestion of pancreatic tissue, the "duct-like" cells were then inoculated into 96-well plates, cloned by limiting dilution, and cultured in DMEM with 20% FBS (see Materials and Methods for a more detailed description). Of the more than 200 isolated clones, six clones were able to be cultured for more than 3 months. One of the clones, named HN#101 (Fig. 1B-a), which formed a flat, monolayer "cobblestone" morphology, was used to evaluate the culture conditions.

Morphology of the Pancreatic Stem Cells Cultured in Different Media

We previously reported that one of the previously isolated pancreatic stem cell clones, the HN#13 cells, could be maintained during culture in DMEM using only 1 of 10 different lots of FBS (19). For efficient isolation and

maintenance of mouse pancreatic stem cells, it is important to identify culture conditions that can be used that are independent of the FBS lot. HN#101 cells were cultured under different culture conditions for their ability to support the growth of pancreatic stem cells. Under culture conditions #1 and #3, the HN#101 cells could be maintained to form a flat "cobblestone" monolayer and continued to divide actively beyond the population doubling level (PDL) 100 (over 3 months) (Fig. 1B-b, B-d). On the other hand, the HN#101 cells formed "fibroblast-like/spindle" structures when grown under culture condition #2 (Fig. 1B-c).

Growth Activity of the Pancreatic Stem Cells

HN#101 cells were evaluated for their growth activity under the three different culture conditions. Under culture conditions #1 and #3, the HN#101 cells divided actively beyond PDL 100 without growth inhibition (Fig. 2). Under culture condition #2, the growth activity of the HN#101 cells gradually decreased (Fig. 2). These data suggest that conditions #1 and #3 are suitable for the culture of mouse pancreatic stem cells.

Gene Expression Profile of the Pancreatic Stem Cells Under Culture Condition #3

To investigate the gene expression in HN#101 cells cultured for 2 months under culture condition #3, an RT-PCR analysis of endodermal/pancreatic progenitor cell markers was performed. Cells that differentiated from ES cells (generated by a stepwise differentiation protocol that relies on intermediates thought to be similar to cell populations present in the developing embryo) (2,5) were used as a positive control. The marker gene expression patterns of the definitive endoderm [sex-determining region Y-box17 (Sox17), forkhead box protein a2 (Foxa2)], gut tube endoderm [hepatocyte nuclear factor 1β (Hnf1β), Hnf4α], and pancreatic progenitors (Hnf6, Pdx1) were detected in HN#101 cells cultured for 2 months under culture condition #3 (Fig. 3). These data suggest that the gene expression pattern in HN#101 cells cultured for 2 months under culture condition #3 was similar to that of undifferentiated HN#101 cells.

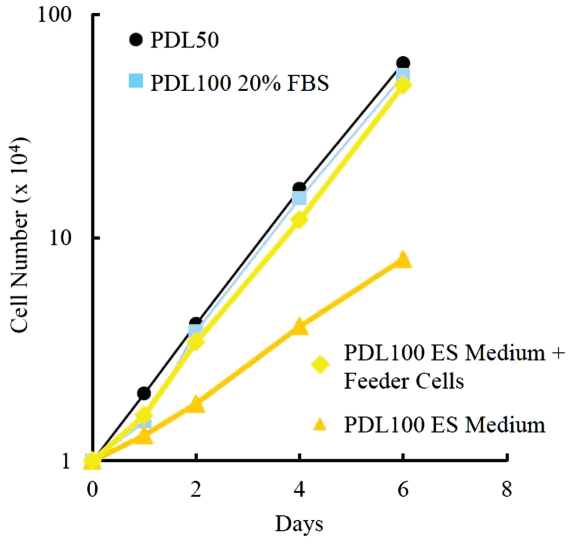

Figure 2. Growth activity of the pancreatic stem cells. HN#101 cells (PDL 50 = control) were evaluated for their growth activity under three different culture conditions (1–3 in Fig. 1). Under culture conditions #1 and #3, the HN#101 cells divided actively beyond PDL 100 without any change in growth activity. Under culture condition #2, the growth activity of the HN#101 cells was gradually decreased.

Differentiation Capacity of the Pancreatic Stem Cells After Culture for 2 Months Under Culture Condition #3

To evaluate whether the differentiation capacity of the pancreatic stem cells was maintained under culture condition #3, the HN#101 cells cultured for 2 months under culture condition #3 were induced to differentiate into insulin-producing cells by treatment with exendin-4, Pdx1 protein, and BETA2/NeuroD protein for 7 to 10 days. We previously reported that Pdx1 and BETA2/NeuroD protein transduction technology could be a safe and valuable strategy for facilitating the differentiation of stem/progenitor cells into insulin-producing cells without requiring the use of gene transfer technology (10,12). The treated cells induced the expression of insulin-2 mRNA (but not insulin-1 mRNA), although the level of insulin-2 mRNA was still low compared with that of primary mouse islets (Fig. 4). We also examined the expression of other transcription factors and pancreas-related genes. After induction, the expression of the BETA2/NeuroD islet-1 (Isl-1) and paired box 6 (Pax6) transcription factors and glucagon were induced (Fig. 4). These data suggest that complete ES cell media and growth of cells on feeder layers, the same culture conditions that are used for mouse ES cells, could be useful in maintaining the undifferentiated state of pancreatic stem cells.

DISCUSSION

In this study, we evaluated the culture conditions required to maintain mouse pancreatic stem cells. The complete ES cell media with 15% FBS and feeder layers of mitomycin C-treated STO cells, the same conditions that are used to culture mouse ES cells, are suitable for culturing mouse pancreatic stem cells. Since the culture in complete ES cell media without feeder cells failed to maintain the undifferentiated state of the mouse pancreatic stem cells, feeder cells appear to be essential for the maintenance of the pancreatic stem cells. On the other hand, we previously reported that the pancreatic stem cells cultured in DMEM with 9 of 10 FBS formed "fibroblast-like" or "spindle" structure and stopped dividing after three to six passages

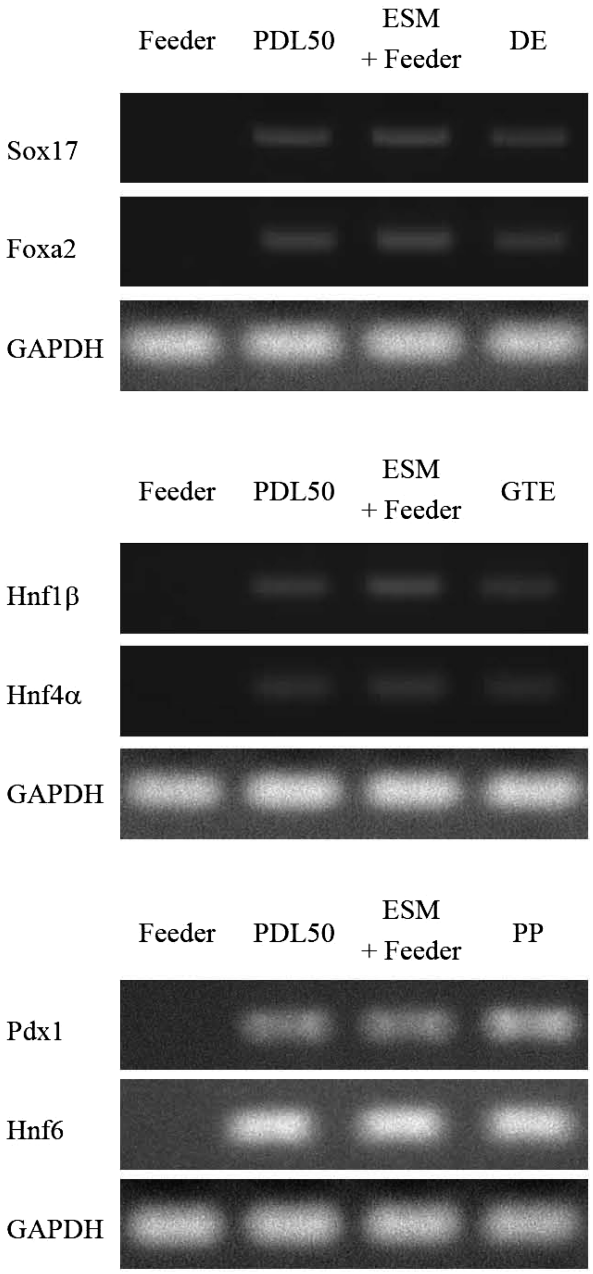

Figure 3. The gene expression profile of the pancreatic stem cells cultured under condition #3. To investigate the gene expression profile of HN#101 cells cultured for 2 months under culture condition #3, an RT-PCR analysis of endodermal/pancreatic progenitor cell markers was performed. Feeder, feeder cells (STO cells, negative control); PDL 50, HN#101 cells at PDL 50 under culture condition #1; ESM+Feeder, HN#101 cells in ES media (ESM) at PDL 100 under culture condition #3; DE, differentiated cells at the definitive endoderm stage derived from ES cells (positive control); GTE, differentiated cells at the gut tube endoderm stage derived from ES cells (positive control); PP, differentiated cells at the pancreatic progenitor stage derived from ES cells (positive control); Sox17, sex-determining region Y box 17; Foxa2, forkhead box A2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Hnf, hepatocyte growth factor; Pdx1, pancreatic and duodenal homeobox factor 1.

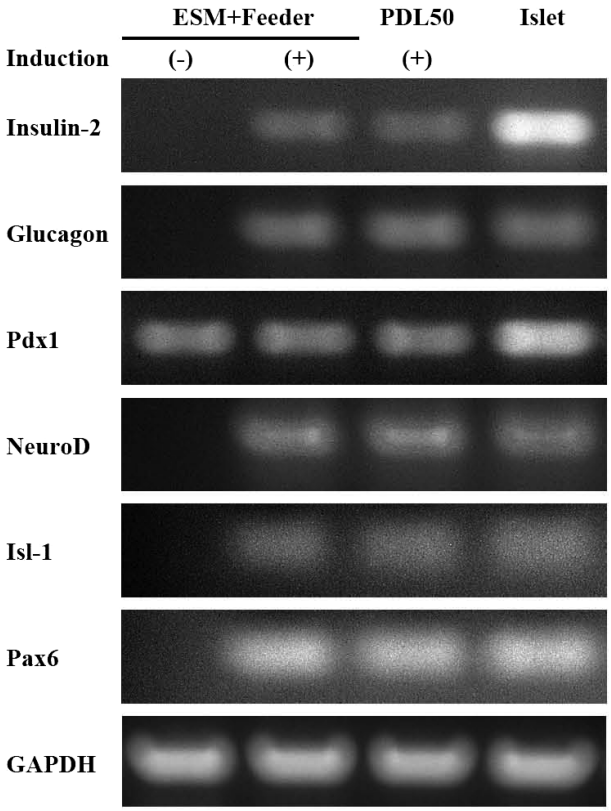

Figure 4. Differentiation capacity of the pancreatic stem cells after culture for 2 months under culture condition #3. To evaluate whether the differentiation capacity of the pancreatic stem cells was maintained under culture condition #3, the HN#101 cells cultured under condition #3 for 2 months were induced to differentiate into insulin-producing cells by treatment with 10 nM exendin-4, 10 mM nicotinamide, 10 ng/ml keratinocyte growth factor (KGF), 100 nM Pdx1 protein, and 100 nM BETA2/NeuroD protein for 7 to 10 days. PCR was performed in a Perkin-Elmer 9700 Thermocycler with 2 μl cDNA (20 ng RNA equivalent) from the treated HN#101 cells. The oligonucleotide primers and cycle number used for the semi-quantitative PCR are shown in Table 1. ESM+Feeder, HN#101 cells at PDL 100 under culture condition #3; PDL 50, HN#101 cells at PDL 50 under culture condition #1; Islet, mouse islets (positive control); Isl-1, islet 1; Pax6, paired homeobox 6.

(19). Although the culture in complete ES cell media without feeder cells failed to maintain the undifferentiated state of the mouse pancreatic stem cells and the growth activity of the pancreatic stem cells gradually decreased, the cells grew beyond PDL 100. These data support that some components in the complete ES cell media may also be important for the maintenance of pancreatic stem cells.

Diabetes is the most prevalent metabolic disease, and the number of diabetic patients worldwide is increasing. One attractive approach for the generation of β-cells involves the expansion and differentiation of adult human pancreatic stem/progenitor cells, which are closely related to the β-cell lineage (1,3,4,7,8,10,12,15,20,22–24). Mouse pancreatic stem

cells have already been isolated, and the culture conditions for the maintenance of these cells have also been determined, as demonstrated in this report. The pancreatic stem cells could be useful in analyzing the molecular mechanisms regulating pancreatic stem/progenitor cell differentiation. Moreover, the techniques used for the isolation and culture of these cells might be useful in the identification and isolation of human pancreatic stem/progenitor cells. Further optimization of the culture conditions is needed to generate insulin-producing cells with glucose sensitivity from these stem/progenitor cells.

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REFERENCES

1. Bonner-Weir, S.; Taneja, M.; Weir, G. C.; Tatarkiewicz, K.; Song, K. H.; Sharma, A.; O'Neil, J. J. In vitro cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. USA 97:7999–8004; 2000.
2. D'Amour, K. A.; Banq, A. G.; Eliazer, S.; Kelly, O. G.; Aqulnick, A. D.; Smart, N. G.; Moorman, M. A.; Kroon, E.; Carpenter, M. K.; Baetqe, E. E. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat. Biotechnol. 24:1392–1401; 2006.
3. Edlund, H. Pancreas: How to get there from the gut? Curr. Opin. Cell Biol. 11:663–668; 1999.
4. Heremans, Y.; Van De Casteele, M.; in't Veld, P.; Gradwohl, G.; Serup, P.; Madsen, O.; Pipeleers, D.; Heimberg, H. Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. J. Cell Biol. 159:303–312; 2002.
5. Kroon, E.; Martinson, L. A.; Kadoya, K.; Banq, A. G.; Kelly, O. G.; Eliazer, S.; Young, H.; Richardson, M.; Smart, N. G.; Cunninqham, J.; Aqulnick, A. D.; D'Amour, K. A.; Carpenter, M. K.; Baetqe, E. E. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat. Biotechnol. 26:443–452; 2008.
6. Kuise, T.; Noguchi, H. Recent progress in pancreatic islet transplantation. World J. Transplant. 1:13–18; 2011.
7. Noguchi, H. Pancreatic stem/progenitor cells for the treatment of diabetes. Rev. Diabet. Stud. 7:105–111; 2010.
8. Noguchi, H. Production of pancreatic beta-cells from stem cells. Curr. Diabetes Rev. 6:184–190; 2010.
9. Noguchi, H. Cell-permeable peptide inhibitor of c-Jun NH2-terminal kinase for the treatment of diabetes. Curr. Bioact. Compd. 6:23–30; 2010.
10. Noguchi, H.; Bonner-Weir, S.; Wei, F. Y.; Matsushita, M.; Matsumoto, S. BETA2/NeuroD protein can be transduced into cells due to an arginine- and lysine-rich sequence. Diabetes 54:2859–2866; 2005.
11. Noguchi, H.; Iwanaga, Y.; Okitsu, T.; Nagata, H.; Yonekawa, Y.; Matsumoto, S. Evaluation of islet transplantation from non-heart beating donors. Am. J. Transplant. 6:2476–2482; 2006.
12. Noguchi, H.; Kaneto, H.; Weir, G. C.; Bonner-Weir, S. PDX-1 protein containing its own antennapedia-like protein transduction domain can transduce pancreatic duct and islet cells. Diabetes 52:1732–1737; 2003.
13. Noguchi, H.; Matsushita, M.; Kobayashi, N.; Levy, M. F.; Matsumoto, S. Recent advances in protein transduction technology. Cell Transplant. 19:649–654; 2010.
14. Noguchi, H.; Matsushita, M.; Okitsu, T.; Moriwaki, A.; Tomizawa, K.; Kang, S.; Li, S. T.; Kobayashi, N.; Matsumoto, S.; Tanaka, K.; Tanaka, N.; Matsui, H. A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. Nat. Med. 10:305–309; 2004.
15. Noguchi, H.; Naziruddin, B.; Jackson, A.; Shimoda, M.; Ikemoto, T.; Fujita, Y.; Chujo, D.; Takita, M.; Kobayashi, N.; Onaca, N.; Hayashi, S.; Levy, M. F.; Matsumoto, S. Characterization of human pancreatic progenitor cells. Cell Transplant. 19:879–886; 2010.
16. Noguchi, H.; Naziruddin, B.; Jackson, A.; Shimoda, M.; Ikemoto, T.; Fujita, Y.; Chujo, D.; Takita, M.; Kobayashi, N.; Onaca, N.; Levy, M. F.; Matsumoto, S. Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation. Transplantation 89:47–54; 2010.
17. Noguchi, H.; Naziruddin, B.; Shimoda, M.; Chujo, D.; Takita, M.; Sugimoto, K.; Itoh, T.; Onaca, N.; Levy, M. F.; Matsumoto, S. A combined continuous density/osmolality gradient for supplemental purification of human islets. Cell Med. 3:33–41; 2012.
18. Noguchi, H.; Naziruddin, B.; Shimoda, M.; Fujita, Y.; Chujo, D.; Takita, M.; Peng, H.; Sugimoto, K.; Itoh, T.; Kobayashi, N.; Onaca, N.; Levy, M. F.; Matsumoto, S. Evaluation of osmolality of density gradient for human islet purification. Cell Transplant. 21:493–500; 2012.
19. Noguchi, H.; Oishi, K.; Ueda, M.; Yukawa, H.; Hayashi, S.; Kobayashi, N.; Levy, M. F.; Matusmoto, S. Establishment of mouse pancreatic stem cell line. Cell Transplant. 18:563–571; 2009.
20. Noguchi, H.; Xu, G.; Matsumoto, S.; Kaneto, H.; Kobayashi, N.; Bonner-Weir, S.; Hayashi, S. Induction of pancreatic stem/progenitor cells into insulin-producing cells by adenoviral-mediated gene transfer technology. Cell Transplant. 15:929–938; 2006.
21. Shapiro, A. M.; Lakey, J. R.; Ryan, E. A.; Korbutt, G. S.; Toth, E.; Warnock, G. L.; Kneteman, N. M.; Rajotte, R. V. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N. Engl. J. Med. 343:230–238; 2000.
22. Street, C. N.; Lakey, J. R.; Shapiro, A. M.; Imes, S.; Rajotte, R. V.; Ryan, E. A.; Lyon, J. G.; Kin, T.; Avila, J.; Tsujimura, T.; Korbutt, G. S. Islet graft assessment in the Edmonton Protocol: Implications for predicting long-term clinical outcome. Diabetes 53:3107–3114; 2004.
23. Suzuki, A.; Nakauchi, H.; Taniguchi, H. Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. Diabetes 53:2143–2152; 2004.
24. Yamamoto, T.; Yamato, E.; Taniguchi, H.; Shimoda, M.; Tashiro, F.; Hosoi, M.; Sato, T.; Fujii, S.; Miyazaki, J. I. Stimulation of cAMP signaling allows isolation of clonal pancreatic precursor cells from adult mouse pancreas. Diabetologia 49:2359–2367; 2006.