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Establishment of new clonal pancreatic β-cell lines (MIN6-K) useful for study of incretin/cAMP signaling

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Abstract

Incretin/cAMP signaling is critical for potentiation of insulin secretion. Although several cell lines of pancreatic β-cells are currently available, there are no cell lines suitable for investigation of incretin/cAMP signaling. In the present study, we have newly established pancreatic β-cell lines (named MIN6-K) from the IT6 mouse, which develops insulinoma. MIN6-K8 cells respond to both glucose and incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), as is the case in pancreatic islets, while MIN6-K20 cells respond to glucose, but not to incretins. Despite the difference in incretin-potentiated insulin secretion between these two cell lines, the accumulation of cAMP after stimulation of GLP-1 is comparable in these cells. Interestingly, we also found that incretin responsiveness is drastically induced by formation of pseudoislets from MIN6-K20 cells to a level comparable to that of pancreatic islets. Thus, these cell lines are useful for studying incretin/cAMP signaling in β-cells.

Key Words: Incretin, cAMP, Pseudoislet

Introduction

Incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released from enteroendocrine cells by ingestion of nutrients, and potentiate insulin secretion in a glucose-dependent manner by activation of cAMP signaling through their specific receptors in the pancreatic β-cell membrane[1]. GLP-1 analogs and dipeptidyl peptidase IV (DPP-IV) inhibitors are currently being used as new hypoglycemic agents to treat patients with type 2 diabetes mellitus (T2DM)[2]. In contrast, it has been reported that GIP is ineffective for treatment of T2DM[3,4], which indicates that GIP receptor-mediated signaling is inactivated in T2DM[5]. Although cAMP is now known to potentiate insulin secretion mediated by both protein kinase A (PKA)-dependent and PKA-independent pathways[6-9], differences in the mechanisms between GLP-1 and GIP signaling in pancreatic β-cells are still unclear. In addition, the nature of incretin-mediated signaling in pancreatic β-cells of T2DM has not been characterized. This is mainly because there is no appropriate system for study of the mechanisms of incretin/cAMP signaling.

Various clonal β-cells are useful models for the study of insulin secretion in pancreatic β-cells. Although several β-cell lines such as RINm5F, HIT, βTC, INS1, and MIN6 have been established[10-14], these cells often exhibit insulin secretory properties different from those of native pancreatic β-cells, and tend to lose glucose-stimulated insulin secretion (GSIS**)** during the course of passages[15,16]. We previously reported that MIN6-m9 cells subcloned from original MIN6 cells retain GSIS after repetitive passages[17]. However, because of their lack of incretin responsiveness, MIN6-m9 cells are not suitable for investigation of incretin/cAMP signaling.

In the present study, we have newly established two pancreatic β-cell lines

(designated MIN6-K8 and MIN6-K20) from the IT6 mouse, which develops insulinoma, and characterized their properties of insulin secretion. We found that these cells show distinct responses to incretins and that formation of pseudoislets drastically induces an incretin responsive state from the unresponsive state.

Materials and Methods

Cloning of MIN6-K cell lines. An IT6 mouse was used to establish pancreatic β-cell lines[14]. Clonal β-cells were obtained by isolating β-cell colonies sprouted on culture dishes of mixed-cells prepared from whole pancreas of an IT6 mouse**,** as previously described[18].

Formation of pseudoislets. Pseudoislets were formed as previously described[16], with slight modifications. Briefly, MIN6-K cells were seeded on dishes coated by 0.1% wt/vol gelatin, and cultured for 7 days in DMEM containing 25 mM glucose.

Measurements of insulin secretion. MIN6-K cells were preincubated for 30 min in HEPES-Krebs buffer[17] with 2.8 mM glucose, and then stimulated for 30 min with various concentrations of glucose in the absence or presence of the incretins for 30 min. Released insulin was measured by insulin assay kit (CIS bio international, Gif sur Yvette, France).

Measurement of cAMP content. MIN6-K cells were incubated for 30 min in the presence or absence of GLP-1 with 16.7 mM glucose. Cellular cAMP levels were determined by using a commercial kit (CIS bio international, Gif sur Yvette, France).

Quantification of mRNA expression. mRNA expressions were quantified by real-time RT-PCR using TaqMan probes (Applied Biosystems, Foster City, CA).

Results

Establishment and characterization of MIN6-K cells. We obtained more than 30 clonal pancreatic β-cell lines from the pancreas of an IT6 mouse. Among these, we selected two cell lines based on their insulin secretory response to glucose and GLP-1. We designated one line MIN6-K8 and the other line MIN6-K20, which were indistinguishable by their morphology (Fig. 1A). MIN6-K8 cells secreted insulin in response to a physiological concentration of glucose and the sulfonylurea glibenclamide. Potentiation of GSIS by incretins (both GLP-1 and GIP) was also evident (Fig. 1B), indicating that the cells resemble native pancreatic islets in terms of the property of insulin secretion. In contrast, while MIN6-K20 cells did respond to glucose and glibenclamide, the cells did not respond to either GLP-1 or GIP (Fig. 1B). Since incretins potentiate insulin secretion through an increase in the intracellular cAMP concentration, we reasoned that the difference in incretin response between MIN6-K8 and -K20 cells might result from a difference in cAMP production. However, this is not the case because the cAMP levels after the addition of GLP-1 in the two cell lines were found to be similar (Fig. 1C). Therefore, signaling distal to cAMP production is likely to be responsible for the different responses to the incretins between MIN6-K8 and MIN6-K20 cells.

Gene expression in MIN6-K cells. We then examined gene expression in MIN6-K8 and -K20 cells by quantitative RT-PCR. Although GSIS in the two cell lines was similar (Fig. 1B), several genes, including GLUT1 and glucokinase, were down-regulated in the incretin non-responsive MIN6-K20 cells (Fig. 2A). The expressions of the receptors for GLP-1 and GIP were not different between MIN6-K8

and -K20 cells. In addition, no significant difference in expressions of adenylyl cyclases, which catalyze ATP to cAMP, and phosphodiesterases, which degrade cAMP, was detected (Fig. 2B).

Normalization of insulin content, basal insulin secretion, and incretin responsiveness by formation of pseudoislets. Although experiments using cell lines are usually performed in monolayer culture conditions, native pancreatic β-cells form three-dimensional structures (islets). We therefore examined insulin secretion in pseudoislets constituted from MIN6-K cells. Pseudoislets, which morphologically resemble native mouse pancreatic islets, were formed on gelatin-coated dishes (Fig. 3A, B and Supporting information Movie 1). Insulin contents in the pseudoislets were drastically increased to levels similar to those of native islets (Fig. 3C), while the expressions of insulin genes were not changed or only slightly increased in pseudoislets (Fig. 3D).

We then investigated insulin secretion in pseudoislets in comparison with that in monolayer-cultured cells (Fig. 4). Insulin secretion from pseudoislets was significantly lower than that from monolayer-cultured cells at a low concentration of glucose. Interestingly, potentiation of GSIS by both GLP-1 and GIP was significantly enhanced in MIN6-K8 cells by formation of pseudoislets. Most strikingly, even incretin non-responsive MIN6-K20 cells, as well as MIN6-m9 cells, clearly responded to both GLP-1 and GIP when pseudoislets were formed. These data demonstrate that these cell lines acquired insulin secretory properties similar to those of native pancreatic islets by formation of pseudoislets.

Discussion

It is well known that incretins such as GIP and GLP-1 activate adenylyl cyclase to increase cAMP in pancreatic β-cells[2]. Elevation of cAMP levels activates both PKA-dependent and PKA-independent pathways, the latter involving Epac2/Rap1 signaling[6-9]. While PKA modulates insulin secretion through phosphorylation of various proteins associated with the secretory process of insulin[8,19], Epac2/Rap1 signaling is also important for potentiation of GSIS by cAMP, probably by increasing the size of a readily releasable pool near the plasma membrane[9,18,20]. However, the link between these pathways and insulin secretion is largely unknown. Although native pancreatic β-cells are an ideal source for investigation of these mechanisms, the limited numbers of pancreatic β-cells that can be isolated from native pancreas hampers the study of incretin/cAMP signaling at the cell level.

We show here that our newly established mouse pancreatic β-cell lines are useful for such study. GLP-1 increased the intracellular cAMP concentration similarly in both incretin responsive MIN6-K8 and incretin non-responsive MIN6-K20 cells, indicating that signals distal to cAMP production differ between the two cell lines. Comparative analysis of MIN6-K8 and -K20 cells should therefore advance understanding the link between cAMP and insulin secretion.

In the present study, we show for the first time that formation of pseudoislets induces incretin responsiveness in pancreatic β-cell lines. Cell-cell adhesion is known to have important roles in cell function and differentiation[21,22]. Cell-cell interactions mediated by gap junctions and/or EphA-ephrin-A[23-25] may participate, at least in part, in the improved insulin secretion in pseudoislets. Pseudoislets constituted from these newly established MIN6-K lines are a useful system for elucidation of incretin/cAMP signaling in β-cells.

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References

1. Elliott RM, Morgan LM, Tredger JA et al. Glucagon-like peptide-1 (7-36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol 1993; 138: 159-66.
2. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007; 132: 2131-57.
3. Nauck MA, Heimesaat MM, Orskov C et al. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993; 91: 301-7.
4. Vilsboll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. Diabetologia 2002; 45: 1111-9.
5. Lynn FC, Pamir N, Ng EH et al. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. Diabetes 2001; 50: 1004-11.
6. Ozaki N, Shibasaki T, Kashima Y et al. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Nat Cell Biol 2000; 2: 805-11.
7. Kashima Y, Miki T, Shibasaki T et al. Critical role of cAMP-GEFII·Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 2001; 276: 46046-53.
8. Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev 2005; 85: 1303-42.
9. Seino S, Takahashi H, Fujimoto W, Shibasaki T. Roles of cAMP signalling in insulin granule exocytosis. Diabetes Obes Metab 2009; 11 Suppl 4: 180-8.
10. Gazdar AF, Chick WL, Oie HK et al. Continuous, clonal, insulin- and

somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. Proc Natl Acad Sci U S A 1980; 77: 3519-23.

11. Santerre RF, Cook RA, Crisel RM et al. Insulin synthesis in a clonal cell line of simian virus 40-transformed hamster pancreatic beta cells. Proc Natl Acad Sci U S A 1981; 78: 4339-43.
12. Efrat S, Linde S, Kofod H et al. Beta-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. Proc Natl Acad Sci U S A 1988; 85: 9037-41.
13. Asfari M, Janjic D, Meda P et al. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 1992; 130: 167-78.
14. Miyazaki J, Araki K, Yamato E et al. Establishment of a pancreatic β cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 1990; 127: 126-32.
15. Praz GA, Halban PA, Wollheim CB et al. Regulation of immunoreactive-insulin release from a rat cell line (RINm5F). Biochem J 1983; 210: 345-52.
16. Hauge-Evans AC, Squires PE, Persaud SJ, Jones PM. Pancreatic β-cell-to-β-cell interactions are required for integrated responses to nutrient stimuli: enhanced Ca^{2+} and insulin secretory responses of MIN6 pseudoislets. Diabetes 1999; 48: 1402-8.
17. Minami K, Yano H, Miki T et al. Insulin secretion and differential gene expression in glucose-responsive and -unresponsive MIN6 sublines. Am J Physiol Endocrinol Metab 2000; 279: E773-81.
18. Shibasaki T, Takahashi H, Miki T et al. Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. Proc Natl Acad Sci U S A 2007; 104: 19333-8.
19. Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation

of insulin secretion from pancreatic β-cells. Endocr Rev 1998; 19: 429-61.

20. Zhang CL, Katoh M, Shibasaki T et al. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. Science 2009; 325: 607-10.
21. Potter E, Bergwitz C, Brabant G. The cadherin-catenin system: implications for growth and differentiation of endocrine tissues. Endocr Rev 1999; 20: 207-39.
22. Minami K, Okano H, Okumachi A, Seino S. Role of cadherin-mediated cell-cell adhesion in pancreatic exocrine-to-endocrine transdifferentiation. J Biol Chem 2008; 283: 13753-61.
23. Bavamian S, Klee P, Britan A et al. Islet-cell-to-cell communication as basis for normal insulin secretion. Diabetes Obes Metab 2007; 9 Suppl 2: 118-32.
24. Konstantinova I, Nikolova G, Ohara-Imaizumi M et al. EphA-Ephrin-A-mediated β cell communication regulates insulin secretion from pancreatic islets. Cell 2007; 129: 359-70.
25. Jaques F, Jousset H, Tomas A et al. Dual effect of cell-cell contact disruption on cytosolic calcium and insulin secretion. Endocrinology 2008; 149: 2494-505.

Figure legends

Fig. 1. Characterization of MIN6-K8 and MIN6-K20 cells. (A) Morphology of K8 and K20 cells. Cells were grown as monolayers on a tissue culture dish in DMEM containing 25 mM glucose. Scale bars, 100 μm. (B) Insulin secretory properties of K8 and K20 cells. Cells were precultured for 2 days in a 96-well plate and preincubated for 30 min in HEPES-Krebs buffer containing 0.1% BSA with 2.8 mM glucose. Incubation was preformed in the presence of the indicated concentrations of glucose or glucose with 10 nM glibenclamide (GLB), 10 nM GLP-1, or 10 nM GIP. Plasma membrane depolarization was induced by addition of 60 mM KCl in the presence of 2.8 mM glucose. (C) cAMP levels in K8 and K20 cells were measured in the presence of 10 nM GLP-1 with 16.7 mM glucose. The amounts of insulin secretion and cAMP content were normalized by the cell DNA content. Data are means ± SEM (n = 4). Unpaired student's *t* test was used for evaluation of statistical significance. $^{**}P < 0.01$.

Fig. 2. Comparison of gene expressions among MIN6-K8, MIN6-K20, and MIN6-m9 cells. (A) Expressions of the genes involved in GSIS. (B) Expressions of the genes encoding incretin receptors, adenylyl cyclases, and phosphodiesterases. The expressions are shown as relative to the level of hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression. Data are means ± SEM (n = 3). Unpaired student's *t* test was used for evaluation of statistical significance between MIN6-K8 and -K20 cells. $^{*}P < 0.05$; $^{**}P < 0.01$. †, undetectable. HK, hexokinase; GK, glucokinase; GLP-1-R, GLP-1 receptor; GIP-R, GIP receptor; ACs, adenylyl cyclases; PDEs, phosphodiesterases.

Fig. 3. Formation of pseudoislets. (A) The course of formation of pseudoislets. Sequential images acquired every 6 h for 7 days are shown. Scale bar, 200 μm. (B) Morphology of pseudoislets and mouse pancreatic islets. Scale bars, 200 μm. (C) Comparison of insulin contents between pseudoislets and monolayer-cultured cells. Data are means ± SEM (n = 4-8). The amount of insulin content was normalized by the cell DNA content. (D) mRNA expression levels of insulin1 and insulin2 in pseudoislets and monolayer-cultured cells. Expressions of these genes are shown as relative to the level of hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression. Data are means ± SEM (n = 3). Unpaired student's *t* test was used for evaluation of statistical significance. $*P < 0.05$; $**P < 0.01$.

Fig. 4. Normalization of insulin secretion in pseudoislets. Pseudoislets and monolayer-cultured cells were preincubated for 30 min with HEPES-Krebs buffer containing 0.1% BSA with 2.8 mM glucose, and incubated for 30 min with 2.8 mM or 16.7 mM glucose with or without 10 nM GLP-1 or 10 nM GIP. The amount of insulin secretion was normalized by the cell DNA content. Data are means ± SEM (n = 8). Unpaired student's *t* test was used for evaluation of statistical significance between monolayer-cultured cells and pseudoislets. $*P < 0.05$; $**P < 0.01$.

Supporting information (SI)

SI. Movie 1. Time-lapse images of pseudoislet formation of MIN6-K20 cells were acquired every 1 h for 7 days by using a BIOREVO BZ-9000 fluorescence microscope (Keyence, Osaka, Japan). Timestamp (h : min : sec) and calibration bar (200 μm) are overlaid on the images.

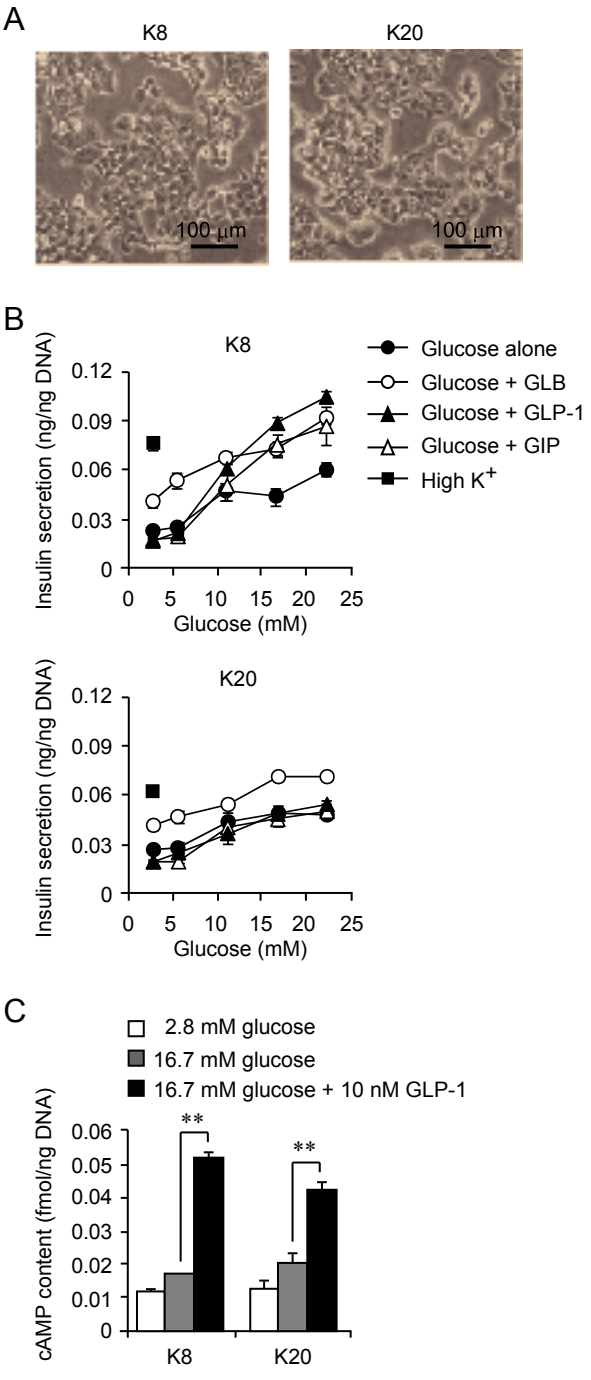
A
K8
K20
100 μm
100 μm
B
K8
Insulin secretion (ng/ng DNA)
0.12
0.09
0.06
0.03
0
0 5 10 15 20 25
Glucose (mM)
Glucose alone
Glucose + GLB
Glucose + GLP-1
Glucose + GIP
High K+
K20
Insulin secretion (ng/ng DNA)
0.12
0.09
0.06
0.03
0
0 5 10 15 20 25
Glucose (mM)
C
2.8 mM glucose
16.7 mM glucose
16.7 mM glucose + 10 nM GLP-1
cAMP content (fmol/ng DNA)
0.06
0.05
0.04
0.03
0.02
0.01
0
**
**
K8
K20

Fig. 1

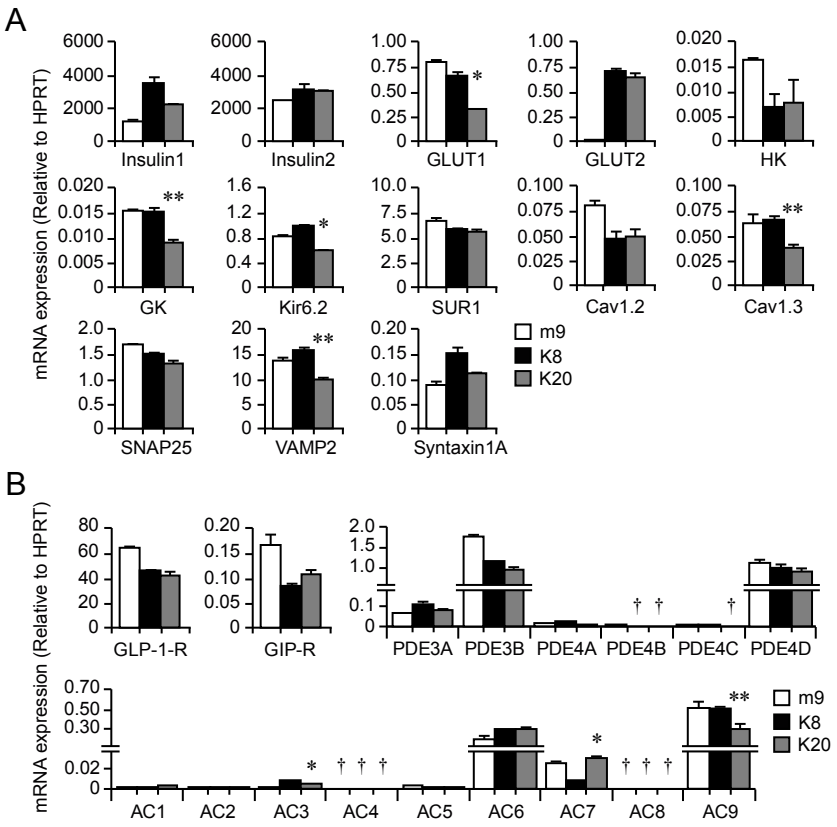
A
mRNA expression (Relative to HPRT)
Insulin1
Insulin2
GLUT1
GLUT2
HK
GK
Kir6.2
SUR1
Cav1.2
Cav1.3
SNAP25
VAMP2
Syntaxin1A
m9
K8
K20
B
GLP-1-R
GIP-R
PDE3A
PDE3B
PDE4A
PDE4B
PDE4C
PDE4D
AC1
AC2
AC3
AC4
AC5
AC6
AC7
AC8
AC9

Fig. 2

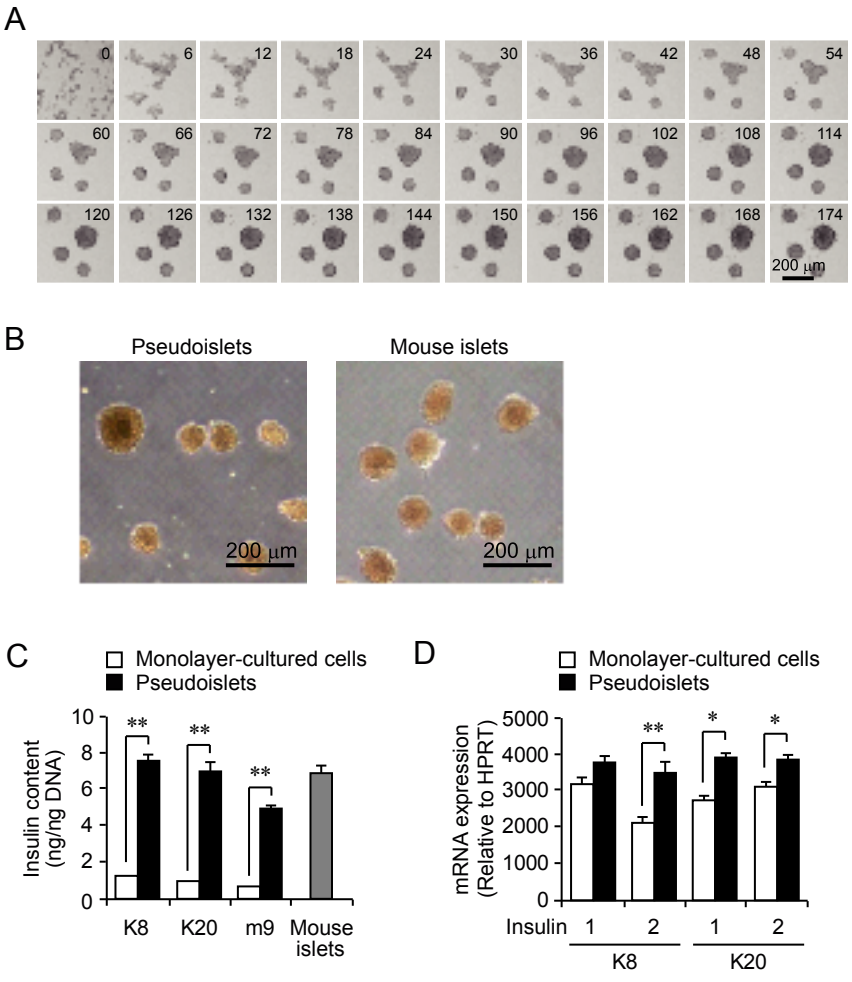
A
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
108
114
120
126
132
138
144
150
156
162
168
174
200 μm
B
Pseudoislets
Mouse islets
200 μm
200 μm
C
Monolayer-cultured cells
Pseudoislets
Insulin content (ng/ng DNA)
10
8
6
4
2
0
**
**
**
K8
K20
m9
Mouse islets
D
Monolayer-cultured cells
Pseudoislets
mRNA expression (Relative to HPRT)
5000
4000
3000
2000
1000
0
**
*
*
Insulin
1
2
1
2
K8
K20

Fig. 3

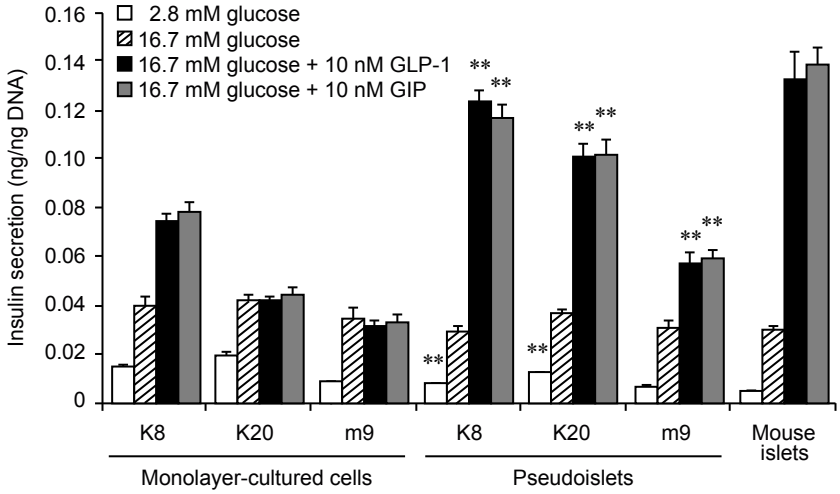
2.8 mM glucose
16.7 mM glucose
16.7 mM glucose + 10 nM GLP-1
16.7 mM glucose + 10 nM GIP
Insulin secretion (ng/ng DNA)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
**
K8
K20
m9
K8
K20
m9
Mouse islets
Monolayer-cultured cells
Pseudoislets

Fig. 4