Biochemical and Biophysical Research Communications 401 (2010) 79–84

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Raldh3 expression in diabetic islets reciprocally regulates secretion of insulin and glucagon from pancreatic islets

Mitsuru Shimamura [a,*,1], Hiroshi Karasawa [b,1], Sachiko Sakakibara [c], Akira Shinagawa [c]

[a] Research and Development Planning Division, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Cardiovascular-Metabolics Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[c] Translational Medicine & Clinical Pharmacology Department, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

ARTICLE INFO

Article history:
Received 24 August 2010
Available online 15 September 2010

Keywords:
Raldh3
Glucose
Insulin
Glucagon
13-*cis* retinoic acid
Diabetes

ABSTRACT

We have previously reported that obesity-induced diabetes developed in high-fat diet (HFD)-fed BDF1 mice. This is caused by insufficient insulin response to an excess glucose load. In this study, we have shown that the enhanced expression of retinaldehyde dehydrogenase 3 (Raldh3) causes functional disorders of pancreatic islets in diabetic mouse models. In the pancreatic islets of HFD-induced diabetic BDF1 mice and spontaneously diabetic C57BL/KsJ$^{db/db}$ mice, gene expression analysis with oligonucleotide microarray revealed a significant increase in Raldh3 expression. Exposure to a culture medium containing a higher glucose concentration (25 mM) significantly increased Raldh3 expression in murine MIN6 and alphaTC1 clone 9 cells, which derived from the α and β-cells of pancreatic islets, respectively. Overexpression of Raldh3 reduced the insulin secretion in MIN6 cells, and surprisingly, increased the glucagon secretion in alphaTC1 clone 9 cells. Furthermore, the knockdown of Raldh3 expression with siRNA decreased the glucagon secretion in alphaTC1 clone 9 cells. Raldh3 catalyzes the conversion of 13-*cis* retinal to 13-*cis* retinoic acid and we revealed that 13-*cis* retinoic acid significantly reduces cell viability in MIN6 and alphaTC1 clone 9 cells, but not in cells of H4IIEC3, 3T3-L1, and COS-1 cell lines. These findings suggest that an increasing expression of Raldh3 deregulates the balanced mechanisms of insulin and glucagon secretion in the pancreatic islets and may induce β-cell dysfunction leading to the development of type 2 diabetes.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Both insulin resistance and β-cell dysfunction are associated with the pathogenesis of type 2 diabetes. It is considered that type 2 diabetes develops when pancreatic islets become incapable of secreting enough insulin to compensate for insulin resistance [1,2]. Obesity is an important risk factor for the development of type 2 diabetes as it induces insulin resistance. We recently reported that obesity-induced diabetes developed in high-fat diet (HFD)-fed BDF1 mice and that it was characterized by severe insulin resistance and significant defects in insulin secretion [3]. The pancreatic islets isolated from HFD-fed BDF1 mice showed a reduced insulin contents and glucose-induced insulin secretion from these islets was also significantly impaired. At the same time, immunohistochemical analysis revealed a significant increase in glucagon-positive cells and glucagon immunoreactivity in the pancreatic islets of HFD-fed BDF1 mice [3]. Expansion of α-cells associated with β-cell loss was also reported in patients with type 2 diabetes [4].

Elevation of glucagon secretion contributes, at least in part, to the hyperglycemia observed in HFD-fed BDF1 mice. Plasma glucagon accelerates hepatic gluconeogenesis and increases fasting glucose levels [5–7]. Furthermore, insulin suppresses glucagon secretion via the insulin receptors in the α-cells of pancreatic islets in normal physiology [8,9]. Thus, the reduction in insulin secretion from pancreatic β-cells and the insulin resistance in α-cells can explain the insufficient suppression of glucagon secretion in HFD-fed BDF1 mice, leading to hyperglycemia.

C57BL/KsJ$^{db/db}$ (*db/db*) mice are susceptible to pancreatic islet exhaustion in insulin-resistant states as compared with C57BL/6J mice [10]. Damore et al. recently reported a study that used oligonucleotide microarray to find the causative genes of reduced insulin secretion in *db/db* mice [11]. We performed comprehensive gene expression analysis of the pancreatic islets of HFD-fed BDF1 mice and revealed significant expression changes in retinaldehyde dehydrogenase 3 (Raldh3). In the present study, we investigated the function of Raldh3 in the pancreatic islets that secrete insulin and glucagon, and examined its relevance in the development of type 2 diabetes.

* Corresponding author. Fax: +81 3 5436 8561.
E-mail address: shimamura.mitsuru.f2@daiichisankyo.co.jp (M. Shimamura).
[1] These authors contributed this work equally.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.09.013

2. Materials & methods

2.1. Materials

2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT), phenazine methosulphate (PMS) and 13-*cis* retinoic acid were purchased from SIGMA (St. Louis, MO). The coding sequence of mouse Raldh3 was amplified by PCR with total RNA extracted from MIN6 cells. The PCR products were cloned into a pENTR/SD-TOPO vector (Invitrogen, Carlsbad, CA) and converted into a pDEST40 vector using the Gateway LR reaction (Invitrogen). The pDEST40 Raldh3 cDNA expression vector was used in the experiments and pDEST40 LacZ gene expression vector was used as a control, which was purchased from Invitrogen. The Validated Stealth RNAi designed to knockdown Raldh3 mRNA was also purchased from Invitrogen. The targeted DNA sequences of Raldh3 and irrelevant Stealth RNAi were as follows: 5′-CCTCTCTCATCAAAGAGGTCGGGTT-3′ (NM_053080, position:715–739); 5′-CCTACTCAACTGAGAGCTGGTCGTT-3′.

2.2. Oligonucleotide microarray analysis and real-time quantitative PCR

The pancreatic islets of BDF1 and *db/db* mice were isolated after collagenase treatment and collected in groups. Total RNA was extracted with TRIzol (Invitrogen) and purified with RNeasy MinElute Cleanup Kit (QIAGEN, Hilden, Germany). Oligonucleotide microarray analysis was performed based on the standard protocol [12]. Biotinylated cRNA was prepared using the Bio Array High Yield RNA Transcript Labeling Kit (ENZO, Farmingdale, NY). The fragmented cRNA was hybridized to a GeneChip Mouse Expression Array 430A and 430B (Affymetrix, Santa Clara, CA) for 16 h at 45 °C. Following post staining procedures, chips were scanned with a GeneChip Scanner 3000 (Affymetrix). The scanned image was quantified using GCOS version 1.1.1 (GeneChip Operating Software, Affymetrix) with the default parameters for the statistical algorithm. One probe set ID (98372_at) was designated as Raldh3 mRNA expression.

To perform real-time quantitative PCR, cDNA was synthesized using the SuperScript Preamplification System (Invitrogen) and then CYBR Green I (Applied Biosystems, Foster City, CA) quantitative PCR (10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C) was performed with the ABI Prism 7700 PCR instrument (Applied Biosystems). Gene specific primers were as follows: 5′-TTGGCCGTCGAGTGTGCTCA-3′ and 5′-TGGCCT CTTCTTGGCGAACT-3′ for mouse Raldh3; 5′-CAGACGCCACTGTCGC TTT-3′ and 5′-TGTCTTTGGAACTTTGTCTGCAA-3′ for mouse cyclophilin.

2.3. Cell culture and transfection assay

We used MIN6 cells reported by Ishihara et al. [13]. AlphaTC1 clone 9 cells were purchased from ATCC. MIN6 cells were cultured and maintained in dulbecco's modified Eagle's medium (DMEM) containing 5.5 mM glucose, 20% fetal calf serum (FCS), 100 μg/mL streptomycin, and 100 units/mL penicillin sulfate. AlphaTC1 clone 9 cells were maintained in DMEM containing 10% FCS. The pDEST40 LacZ and Raldh3 cDNA expression vectors were transfected into MIN6 and alphaTC1 clone 9 cells cultured in a 24-well plate using Lipofectamine 2000 (Invitrogen). The transfected cells were cultured for 48 h and were used in the experiments. After washing with Krebs buffer containing 0.2% BSA, the treated cells were incubated in 0.2% BSA Krebs buffer containing 5.5 or 25 mM glucose for 1 or 2 h. The culture supernatants of MIN6 and alphaTC1 clone 9 cells were collected for determination of insulin and glucagon levels, respectively. In some experiments, total RNA was extracted and gene expression levels were analyzed.

2.4. Animal studies and the measurements of insulin and glucagon

Male BDF1 mice aged 6 weeks were purchased from Charles River Japan (Yokohama, Japan). BDF1 mice were fed a regular chow diet (Chow) or HFD [3] for 16 weeks and HFD-fed BDF1 mice exhibiting obesity and diabetes and Chow-fed control mice were sacrificed for gene expression analysis. Male KK mice were purchased from CLEA Japan (Tokyo, Japan) and used in experiments at 6–7 weeks of age. Male *db/db* mice were purchased from Charles River Japan and used at 5–8 weeks of age. Mice were housed in a temperature- and humidity-controlled facility with free access to water and food. Blood samples were collected from the tail vein. Plasma glucose levels were measured using Glucose CII-Test Wako kit (Wako Chemicals, Osaka, Japan). Insulin levels in plasma and in the MIN6 cell culture media were measured using Ultrasensitive Insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). Glucagon levels in plasma and in the culture media of alphaTC1 clone 9 cells were measured by an enzyme immunoassay kit (YM090, Yanaihara Institute, Japan). All animal experiments were carried out in accordance with the guidelines provided by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. (Tokyo, Japan).

2.5. Cell viability assay

Cell viability was measured by an XTT-based colorimetric assay [14]. Cells were seeded in a 96-well plate at concentration of 1×10^4 cells per well and cultured in DMEM supplemented with different concentrations of 13-*cis* retinoic acid for 24 h. XTT and PMS media were applied to the wells with final concentrations of 0.5 mM and 8.2 μM, respectively, and the cells were incubated for another 2 h at 37 °C. Absorbance at 450 nm was measured and cell viabilities were calculated.

2.6. Statistical analysis

All data are expressed as the mean ± SEM. Statistical significance of differences between groups was tested by Student's *t*-test. A P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. High glucose levels enhanced Raldh3 mRNA expression in pancreatic islets and the islet-derived cells

To isolate novel genes related to the HFD-induced reduction of insulin secretion, we evaluated the gene expression in pancreatic islets of Chow- or HFD-fed BDF1 mice using oligonucleotide microarray (another new manuscript in preparation). After 16 weeks of feeding, the body weights of the Chow- and HFD-fed groups were 39.7 ± 1.8 versus 53.8 ± 0.5 g and the blood glucose was 183 ± 9 versus 389 ± 11 mg/dL (Fig. 1A). Interestingly, the signal values of the probe set ID designated as Raldh3 mRNA expression were about 7 times higher in HFD-fed BDF1 mice than those in Chow-fed BDF1 mice (Fig. 1A).

Next, we analyzed Raldh3 expression in the pancreatic islets of another diabetic model. Severe diabetes associated with obesity spontaneously developed in most of *db/db* mice at 5 weeks of age. Mean body weights at 5 and 7 weeks of age were

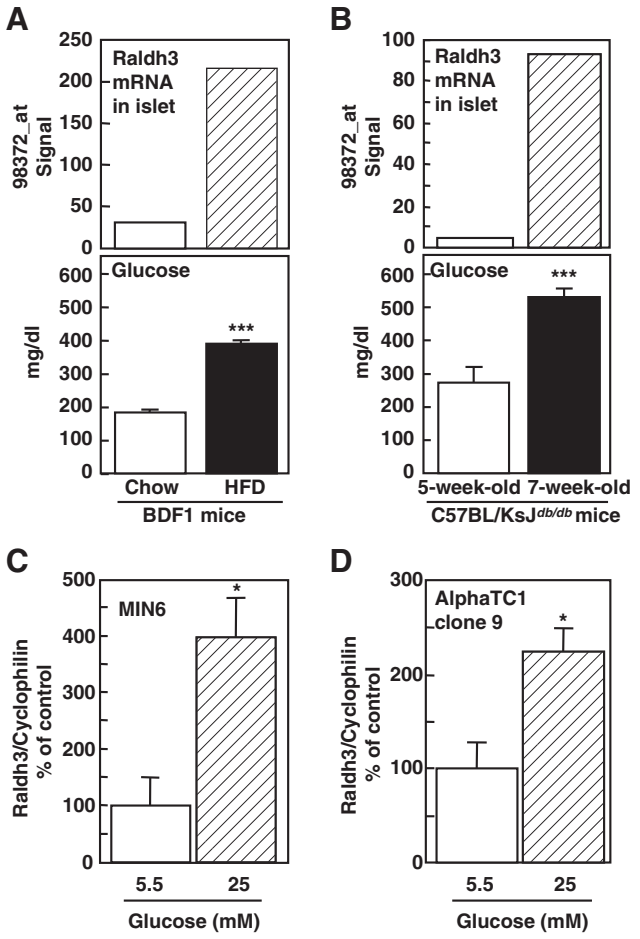

Fig. 1. Elevated Raldh3 mRNA expression in diabetic mouse pancreatic islets and the islet-derived cell lines cultured in high glucose conditions. (A) Raldh3 expression levels in pancreatic islets and plasma glucose levels of Chow- (open columns) or HFD-fed BDF1 mice (closed columns). Raldh3 expression levels (signals) were measured in pooled samples and plasma glucose levels are shown as the mean ± SEM of three mice. (B) Raldh3 expression levels and plasma glucose levels in *db/db* mice at 5 weeks of age (open columns) and 7 weeks of age (closed columns). Data derived from 10 animals per group. $^{***}P < 0.001$ versus Chow or 5-week-old. The signal values of the probe set ID (98372_at) designated as Raldh3 mRNA expression are shown. (C and D) Raldh3 expression in MIN6 and alphaTC1 clone 9 cells cultured with 5.5 (control, open columns) or 25 mM glucose (hatched columns). Raldh3 expression levels were normalized by cyclophilin expression and shown as percent of control. Data are the mean ± SEM of three assays. $^{*}P < 0.05$ versus 5.5 mM glucose.

25.3 ± 1.1 versus 35.4 ± 0.5 g and blood glucose levels were 274 ± 46 versus 531 ± 25 mg/dL. In the pancreatic islets, the signal values of the probe set ID designated as Raldh3 expression at 7 weeks of age was about 20 times as high as that at 5 weeks of age (Fig. 1B). These observations indicate that Raldh3 expression generally increases in the pancreatic islets of obesity-associated diabetic mice.

Next, we examined the effects of direct exposure to high glucose on Raldh3 gene expression in cultured cells derived from murine pancreatic islets. Insulin-producing MIN6 cells and glucagon-producing alphaTC1 clone 9 cells were cultured under normal (5.5 mM) and high (25 mM) glucose conditions, and the expression of Raldh3 was examined. Interestingly, both MIN6 and alphaTC1 clone 9 cells showed elevated Raldh3 expression in 25 mM glucose condition (100 ± 50% versus 397 ± 71%, $P < 0.05$; 100 ± 29% versus 224 ± 25%, $P < 0.05$) (Fig. 1C and D). These *in vitro* and *in vivo* results indicate that hyperglycemia elevates Raldh3 expression in the pancreatic islets.

3.2. Change in Raldh3 expression modulates insulin and glucagon secretion in vitro

MIN6 cells were used to study the effect of changes in Raldh3 expression on insulin secretion. MIN6 cells transfected with a Raldh3 expression vector showed significantly decreased insulin secretion (LacZ versus Raldh3, 100 ± 7% versus 29 ± 6%, $P < 0.001$) (Fig. 2A). Using alphaTC1 clone 9 cells, we studied the effect on glucagon secretion. Interestingly, alphaTC1 clone 9 cells transfected with the Raldh3 expression vector showed significantly increased glucagon secretion (LacZ versus Raldh3, 100 ± 15% versus 212 ± 33%, $P < 0.05$) (Fig. 2B). Next, we transfected alphaTC1 clone 9 cells with Stealth RNAi specific to Raldh3 (Raldh3 siRNA) and found that Raldh3 expression was reduced to 35% compared with that by irrelevant siRNA transfection (Fig. 2C). In this condition, glucagon secretion was significantly decreased (irrelevant siRNA versus Raldh3 siRNA, 100 ± 8% versus 51 ± 12%, $P < 0.05$) (Fig. 2D). These results suggest that Raldh3 decreases insulin secretion from pancreatic β-cells, whereas it increases glucagon secretion from pancreatic α-cells.

3.3. Observation of insulin, glucagon, and glucose levels in obesity-associated type 2 diabetes mouse models

The results described above suggest that Raldh3 expression is closely associated with type 2 diabetes. To reinforce this hypothesis, we observed the development of obesity-induced diabetes in

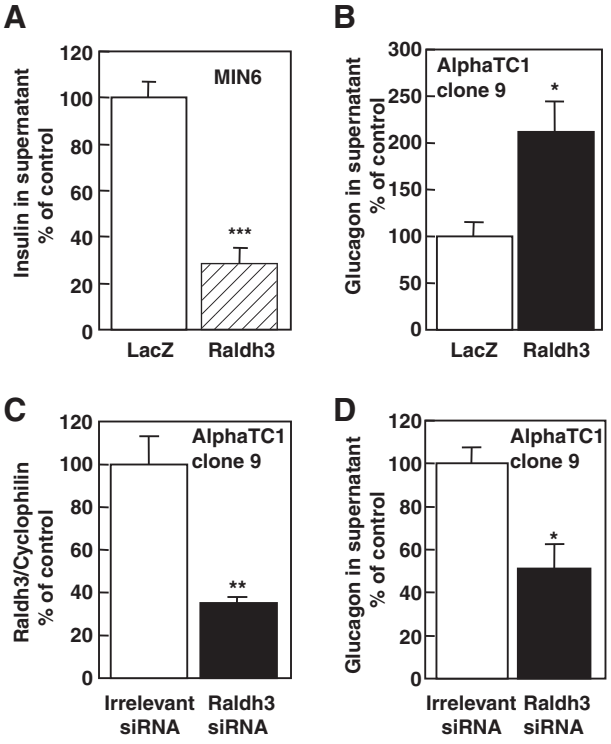

Fig. 2. Effects of Raldh3 expression changes on insulin and glucagon secretion in MIN6 and alphaTC1 clone 9 cells. (A) Insulin secretion from MIN6 cells transfected with LacZ (control, open column) or Raldh3 (hatched column) expression vector. (B) Glucagon secretion from alphaTC1 clone 9 cells transfected with LacZ or Raldh3 expression vector. (C) Raldh3 expression in alphaTC1 clone 9 cells transfected with irrelevant siRNA (control, open column) or Raldh3 siRNA (closed column). Raldh3 expression levels were normalized by cyclophilin expression. (D) Glucagon secretion in the alphaTC1 clone 9 cells transfected with irrelevant or Raldh3 siRNA. Data are the mean ± SEM of 3–4 assays and are shown as percent of control. $^{*}P < 0.05$ versus LacZ or irrelevant siRNA; $^{**}P < 0.01$ versus irrelevant siRNA; $^{***}P < 0.001$ versus LacZ.

db/db and KK mice and examined its association with hormonal changes. In both models, hyperinsulinemia preceded hyperglycemia. When plasma insulin levels started to drop, hyperglycemia developed (Fig. 3A and B).

In *db/db* mice, plasma glucose levels were in a normal range at 5 weeks of age and increased thereafter along with a decline of plasma insulin levels (Fig. 3A). This observation coincided with the elevation of Raldh3 expression in the pancreatic islets as shown in Fig. 1B. Although plasma glucagon levels were almost unchanged in these mice, the steep increase in the plasma glucagon/insulin ratio, which was reported as the cause of hyperglycemia observed in *db/db* mice [15], could be partly derived from the increase in Raldh3 expression.

In normal physiology, insulin negatively regulates glucagon secretion and insulin levels show a negative correlation with glucagon levels [16]. In KK mice, plasma glucagon levels continuously decreased until day 9, opposing the increase in insulin levels; plasma glucose levels were maintained at a normal level during this period (Fig. 3B, open symbols). However, on day 10, stagnation of hyperinsulinemia and the backward increase of glucagon occurred, suggesting a breakdown of normal physiology, and glucose levels increased (Fig. 3B, filled symbols). This observation suggests the importance of plasma glucagon levels in the development of hyperglycemia.

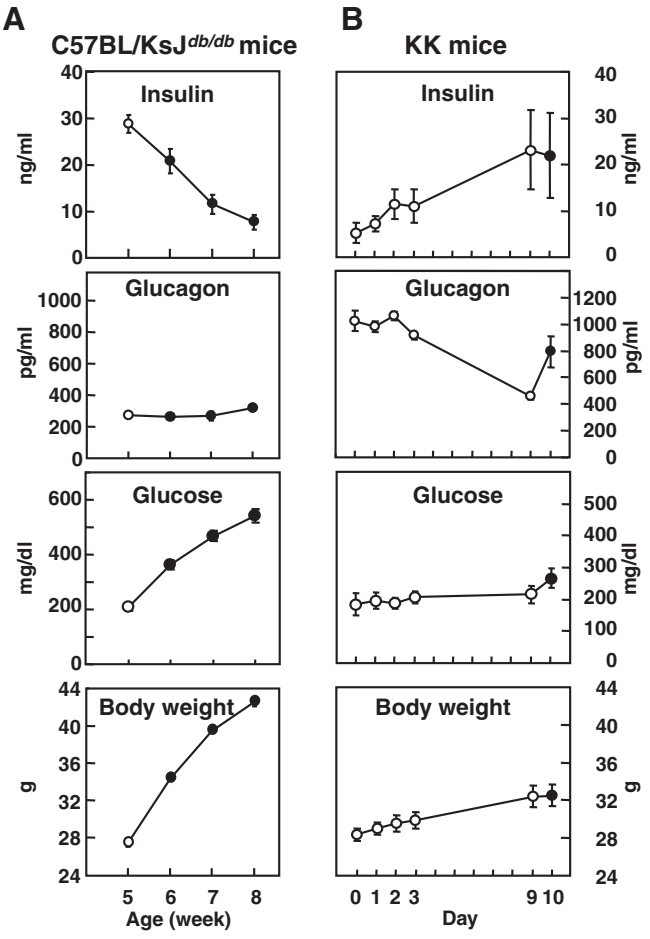

Fig. 3. Observation of the development of obesity-associated diabetes in different mouse models of type 2 diabetes. Plasma insulin, glucagon, and glucose levels and body weight were monitored in *db/db* mice and KK mice. (A) Parameters measured in *db/db* mice from 5 to 8 weeks of age. (B) Parameters measured in KK mice for 10 days from 6 to 7 weeks of age. Open and filled symbols indicate that mice were normoglycemic and hyperglycemic state, respectively, at each point examined. Data are the mean ± SEM of four mice.

3.4. 13-cis retinoic acid lowered the cell viabilities of MIN6 and alphaTC1 clone 9

The Raldh1-4 family enzymes catalyze the conversion of 13-*cis* retinal to 13-*cis* retinoic acid [17]. Thus, increased Raldh3 expression associated with diabetes progression can lead to an accumulation of retinoic acid in pancreatic islet cells. To examine the possible proliferative effects of retinoic acid, we measured cell viabilities in cells cultured with 13-*cis* retinoic acid. Surprisingly, 13-*cis* retinoic acid lowered the cell viabilities of MIN6 cells in a dose dependent manner (72 ± 1% reduction at 10 μM, $P < 0.01$ versus control; Fig. 4A). Furthermore, 13-*cis* retinoic acid also lowered the cell viabilities of alphaTC1 clone 9 cells (73 ± 2% and 77 ± 1% reduction at 1 and 10 μM, respectively, $P < 0.001$ versus control; Fig. 4B). We also evaluated the effects of 13-*cis* retinoic acid in H4IIEC3, 3T3-L1 and COS-1 cells, cell lines that do not originate from the pancreatic islets. However, none of these cells showed alterations in cell viability up to 10 μM (Fig. 4C–E). As far as we investigated, the pancreatic islet-derived cells, MIN6 and alphaTC1 clone 9, were specifically sensitive to the toxicity of 13-*cis* retinoic acid. These results suggest that the possible accumulation of retinoic acid resulting from enhanced Raldh3 expression may lead to the dysfunction of the pancreatic islets.

4. Discussion

In the present study, we have revealed that Raldh3 expression is highly up-regulated in the pancreatic islets of type 2 diabetes model mice by performing a comprehensive gene expression analysis, and have shown that Raldh3 expression and retinoid metabolisms in the pancreatic islets have a significant effect on the balanced control of glucagon and insulin secretion.

Our data suggest that enhanced Raldh3 expression and altered retinoid metabolism in pancreatic islet cells are associated with the pathogenesis of type 2 diabetes. First, enhanced Raldh3 expression was commonly observed in two different diabetes models, HFD-fed BDF1 mice and *db/db* mice, regardless of their differences in etiological differences. Furthermore, a similar phenomenon was observed in islet-derived cells cultured in a high glucose condition. It was found that overexpression of Raldh3 increased glucagon secretion from alpha TC1 clone 9 cells while it decreased insulin secretion from MIN6 cells, which would exacerbate glucose metabolism if it occurred in the pancreatic islets of diabetic mice. In fact, concomitant hormone changes and the development of hyperglycemia were observed in *db/db* mice and KK mice. Finally, the vulnerability of MIN6 and alpha TC1 clone 9 cells to 13-*cis* retinoic acid, which can be produced by a Raldh3-catalyzed reaction, suggests that a possible elevation of 13-*cis* retinoic acid in the pancreatic islets may play an important role in inducing β-cell dysfunction.

To our knowledge, this is the first report on an association between Raldh3 and diabetes or insulin and glucagon secretion from pancreatic islets. Previously, Raldh3 has been reported to be expressed in the developing retina, forebrain, and pituitary gland, which generate retinoic acids [18–20]. However, because homozygous knockout mice of Raldh3 die shortly after birth [21], its physiological role in a developed stage still remains unclear. Our data strongly suggest the importance of retinoid metabolism in the pancreatic islets.

An association between retinoid metabolism and pancreatic endocrine cells has previously been suggested at least in a developmental stage. Raldh2 is expressed in the dorsal pancreatic mesenchyme at the early stage of pancreas specification and it is reported that early glucagon-expressing cells do not develop in Raldh2 knockout embryos [22]. It is highly possible that retinoids play a

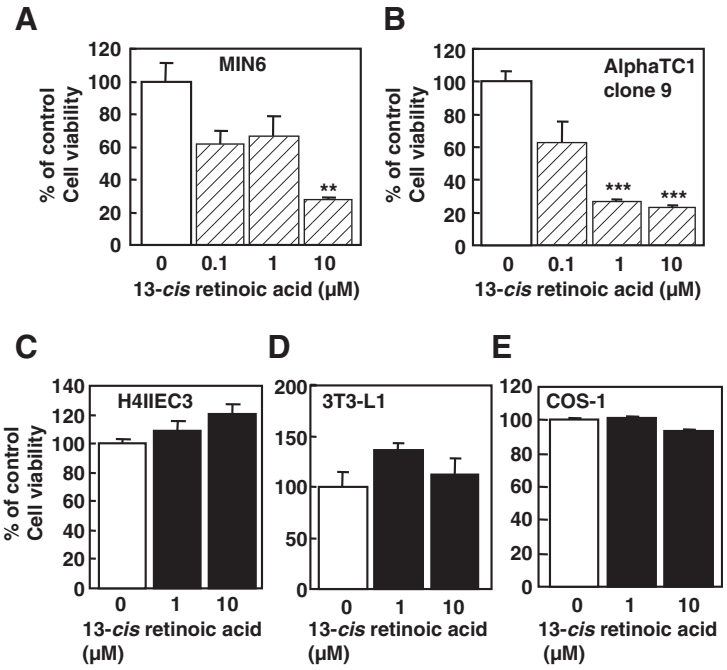

Fig. 4. Reduction of cell viabilities in MIN6 and alphaTC1 clone 9 cells cultured with 13-*cis* retinoic acid. (A) MIN6, (B) alphaTC1 clone 9, (C) H4IIEC3, (D) 3T3-L1, and (E) COS-1 cells were treated with 13-*cis* retinoic acid in serum-free DMEM for 24 h and cell viabilities were measured. Cell viabilities are shown as percent of control (0 μM, open columns). Data are the mean ± SEM of 3 assays. $^{**}P < 0.01$ or $^{***}P < 0.001$ versus control.

role in preservation of pancreatic endocrine cells in adult tissue and Raldh3 modifies cell viability by modulating retinoid metabolism.

On the other hand, Raldh1 has been reported to be associated with metabolic diseases. Knockout mice of Raldh1 resisted diet-induced obesity and insulin resistance and showed increased energy dissipation [23]. Raldh1 is expressed in the adipose tissue and is suggested to alter lipogenesis by modulating retinoid metabolism in the adipose tissue. In this study, we focused on the retinoid metabolism in the pancreatic islets. According to our hypothesis, Raldh3 exacerbates obesity-induced disorders of glucose metabolism by catalyzing retinaldehyde to retinoic acid. At present, it is still unclear which of decreased retinaldehyde or increased retinoic acid mediated by increased Raldh3 activity impairs the normal function of pancreatic islets. However, the observation that retinoic acid decreases cell viability of MIN6 and alphaTC1 clone 9 cells suggests possible negative effects of retinoic acid generated by Raldh3 in the pancreatic islets.

In summary, this study shows that Raldh3 expression in the pancreatic islets significantly increases under hyperglycemic conditions. Enhanced Raldh3 expression decreases insulin secretion and increases glucagon secretion from pancreatic β-cells and α-cells, respectively. Accumulated retinoic acid in the pancreatic islets may precede the dysfunction of the islets.

Acknowledgments

We also thank Dr. Jun Ohsumi, Dr. Takafumi Kohama and Dr. Ichiro Kawashima for fruitful discussion.

References

[1] S.E. Kahn, R.L. Hull, K.M. Utzschneider, Mechanisms linking obesity to insulin resistance and type 2 diabetes, Nature 444 (2006) 840–846.
[2] P.J. Guillausseau, T. Meas, M. Virally, et al., Abnormalities in insulin secretion in type 2 diabetes mellitus, Diabetes. Metab. 34 (Suppl. 2) (2008) S43–48.
[3] H. Karasawa, S. Nagata Goto, K. Takaishi, et al., A novel model of type 2 diabetes mellitus based on obesity induced by high-fat diet in BDF1 mice, Metabolism 58 (2009) 296–303.
[4] K.H. Yoon, S.H. Ko, J.H. Cho, et al., Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea, J. Clin. Endocrinol. Metab. 88 (2003) 2300–2308.
[5] P.E. Cryer, Glucagon and hyperglycaemia in diabetes, Clin. Sci. (Lond.) 114 (2008) 589–590.
[6] K.W. Sloop, M.D. Michael, J.S. Moyers, Glucagon as a target for the treatment of Type 2 diabetes, Expert. Opin. Ther. Targets. 9 (2005) 593–600.
[7] C.G. Fanelli, F. Porcellati, P. Rossetti, et al., Glucagon: the effects of its excess and deficiency on insulin action, Nutr. Metab. Cardiovasc. Dis. 16 (Suppl. 1) (2006) S28–34.
[8] C.J. Greenbaum, P.J. Havel, G.J. Jr Taborsky, et al., Intra-islet insulin permits glucose to directly suppress pancreatic A cell function, J. Clin. Invest. 88 (1991) 767–773.
[9] D. Kawamori, A.J. Kurpad, J. Hu, et al., Insulin signaling in alpha cells modulates glucagon secretion in vivo, Cell. Metab. 9 (2009) 350–361.
[10] K.P. Hummel, D.L. Coleman, P.W. Lane, The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains, Biochem. Genet. 7 (1972) 1–13.
[11] A.A. Anderson, J. Helmering, T. Juan, et al., Pancreatic islet expression profiling in diabetes-prone C57BLKS/J mice reveals transcriptional differences contributed by DBA loci, including Plagl1 and Nnt, Pathogenetics 2 (2009) 1.
[12] S. Uryu, S. Tokuhiro, T. Oda, Beta-Amyloid-specific upregulation of stearoyl coenzyme A desaturase-1 in macrophages, Biochem. Biophys. Res. Commun. 303 (2003) 302–305.
[13] H. Ishihara, T. Wada, N. Kizuki, et al., Enhanced phosphoinositide hydrolysis via overexpression of phospholipase C beta1 or delta1 inhibits stimulus-induced insulin release in insulinoma MIN6 cells, Biochem. Biophys. Res. Commun. 254 (1999) 77–82.
[14] J. Diao, Z. Asghar, C.B. Chan, et al., Glucose-regulated glucagon secretion requires insulin receptor expression in pancreatic alpha-cells, J. Biol. Chem. 280 (2005) 33487–33496.
[15] H. Kodama, M. Fujita, M. Yamazaki, et al., The possible role of age-related increase in the plasma glucagon/insulin ratio in the enhanced hepatic gluconeogenesis and hyperglycemia in genetically diabetic (C57BL/KsJ-db/db) mice, Jpn. J. Pharmacol. 66 (1994) 281–287.
[16] G. Jiang, B.B. Zhang, Glucagon and regulation of glucose metabolism, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E671–678.
[17] C.E. Graham, K. Brocklehurst, R.W. Pickersgill, et al., Characterization of retinaldehyde dehydrogenase 3, Biochem. J. 394 (2006) 67–75.
[18] F.A. Mic, A. Molotkov, X. Fan, et al., RALDH3, a retinaldehyde dehydrogenase that generates retinoic acid, is expressed in the ventral retina, otic vesicle and olfactory pit during mouse development, Mech. Dev. 97 (2000) 227–230.

[19] N. Molotkova, A. Molotkov, G. Duester, Role of retinoic acid during forebrain development begins late when Raldh3 generates retinoic acid in the ventral subventricular zone, Dev. Biol. 303 (2007) 601–610.
[20] K. Fujiwara, F. Maekawa, M. Kikuchi, et al., Expression of retinaldehyde dehydrogenase (RALDH)2 and RALDH3 but not RALDH1 in the developing anterior pituitary glands of rats, Cell. Tissue. Res. 328 (2007) 129–135.
[21] V. Dupe, N. Matt, J.M. Garnier, et al., A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment, Proc. Natl. Acad. Sci. USA 100 (2003) 14036–14041.
[22] M. Martin, J. Gallego Llamas, V. Ribes, et al., Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice, Dev. Biol. 284 (2005) 399–411.
[23] O. Ziouzenkova, G. Orasanu, M. Sharlach, et al., Retinaldehyde represses adipogenesis and diet-induced obesity, Nat. Med. 13 (2007) 695–702.