Aristaless-Related Homeobox Plays a Key Role in Hyperplasia of the Pancreas Islet α–Like Cells in Mice Deficient in Proglucagon-Derived Peptides

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Abstract

Defects in glucagon action can cause hyperplasia of islet α-cells, however, the underlying mechanisms remain largely to be elucidated. Mice homozygous for a glucagon-GFP knock-in allele (Gcg<sup>gfp/gfp</sup>) completely lack proglucagon-derived peptides and exhibit hyperplasia of GFP-positive α-like cells. Expression of the transcription factor, aristaless-related homeobox (ARX), is also increased in the Gcg<sup>gfp/gfp</sup> pancreas. Here, we sought to elucidate the role of ARX in the hyperplasia of α-like cells through analyses of two Arx mutant alleles (Arx<sup>P355L/Y</sup> and Arx<sup>330insGCG/7/Y</sup>) that have different levels of impairment of their function. Expression of Gfp and Arx genes was higher and the size and number of islets increased in the Gcg<sup>gfp/gfp</sup> pancreas compared to and Gcg<sup>gfp/gfp</sup> mice at 2 weeks of age. In male Gcg<sup>gfp/gfp</sup> mice that are hemizygous for the Arx<sup>P355L/Y</sup> mutation that results in a protein with a P355L amino acid substitution, expression of Gfp mRNA in the pancreas was comparable to that in control Gcg<sup>gfp/gfp</sup>/Arx<sup>+/Y</sup> mice. The increases in islet size and number were also reduced in these mice. Immunohistochemical analysis showed that the number of GFP-positive cells was comparable in Gcg<sup>gfp/gfp</sup>/Arx<sup>P355L/Y</sup> and Gcg<sup>gfp/gfp</sup>/Arx<sup>+/Y</sup> mice. These results indicate that the hyperplasia is reduced by introduction of an Arx mutation. Arx<sup>P355L/Y</sup> mice appeared to be phenotypically normal; however, Arx<sup>330insGCG/7/Y</sup> mice that have a mutant ARX protein with expansion of the polyalanine tract had a reduced body size and shortened life span. The number of GFP positive cells was further reduced in the Gcg<sup>gfp/gfp</sup>/Arx<sup>330insGCG/7/Y</sup> mice. Taken together, our findings show that the function of ARX is one of the key modifiers for hyperplasia of islet α-like cells in the absence of proglucagon-derived peptides.

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Introduction

Multiple bioactive peptides, including glucagon and glucagon-like peptides (GLPs) are produced through cell-type specific cleavage of proglucagon, which is encoded by the glucagon gene (Gcg) [1,2,3]. In order to gain insights into the physiological function of proglucagon-derived peptides, we recently generated Gcg-GFP (green fluorescent protein) knock-in mice (Gcg<sup>gfp/gfp</sup>). Homozygous Gcg<sup>gfp/gfp</sup> mice lack all proglucagon-derived peptides and develop prominent hyperplasia of islet α-like cells, which are GFP-positive but do not contain glucagon; by contrast, hyperplasia of intestinal L-like cells, which are also GFP-positive but do not contain GLPs, was not observed [4]. We also found that hyperplasia of α-like cells was associated with a marked increase in the level of Aristaless-related homeobox (Arx) mRNA [4].

Arx is a homeobox gene that is expressed in the central nervous system and plays an important role in brain development [5]. The gene is located on the X-chromosome in both humans and mice, and mutations of the gene cause severe X-linked neurological disorders in humans [6,7]. ARX also plays a pivotal role in the development of pancreatic islet α-cells; Arx-null mice fail to develop mature islet α-cells with a concomitant increase in the numbers of β- and δ-cells [8]. Conversely, forced expression of ARX in mature β-cells promotes a conversion of the cells into glucagon-producing cells [9]. The present study was aimed to characterize the role of Arx in the hyperplasia of GFP-positive, α-like cells in the pancreatic islets of Gcg<sup>gfp/gfp</sup> mice.

Arx null mice die at 2 days after birth [8], and therefore cannot be used for analyzing postnatal hyperplasia of α-like cells in the Gcg<sup>gfp/gfp</sup> mice [4]. To avoid this difficulty, we obtained two mouse strains with partial defects in ARX functions: one strain has elongation of GCG-triplet repeats, which encode the polyalanine tract (330ins[GCG]7); the other strain has an amino acid substitution (P355L/GCG/Tyr) [10]. Functional impairment of ARX-330ins[GCG]7 is more severe than ARX-P355/353L, as the Arx<sup>330insGCG/7</sup> mice exhibit greater neurological abnormalities than Arx<sup>P355L/Y</sup> mice [10]. Homologous human mutations have been identified in patients with X-linked mental retardation: a homologue of Arx<sup>330insGCG/7</sup>, hereafter referred to as Arx-7, was identified in patients with X-linked infantile spasms syndrome/West syndrome, and a homologue of Arx-P355/353L, hereafter referred to as Arx-
PLs, was found in patients with X-linked myoclonic epilepsy with generalized spasticity and intellectual disability [10]. Through crosses between Arx mutant mice and Gcg<sup>+/+</sup> mice, we generated 6 different male mutant genotypes: Gcg<sup>+/-Arx<sup>+/+</sup></sup>, Gcg<sup>+/-Arx<sup>PL/ PL</sup></sup>, Gcg<sup>+/+Arx<sup>-/-</sup></sup>, Gcg<sup>+/+Arx<sup>-PL/PL</sup></sup>, Gcg<sup>+/+Arx<sup>-/-</sup></sup>, and Gcg<sup>+/+Arx<sup>-/-</sup></sup>. These mice were used in the analyses described below.

Materials and Methods

Animals

We generated heterozygous Glucagon-GFP knock-in (Gcg<sup>+/+</sup>) mice as described previously and backcrossed the mice to the C57BL/6J strain for more than 10 generations [11]. Females carrying an Arx mutation (Arx<sup>PL/PL</sup> or Arx<sup>-/-</sup>) on a C57BL/6J genetic background [10] were obtained from the Experimental Animal Division, RIKEN Bioresource Center and mated with male Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice. Arx/Glucagon double heterozygous females, Gcg<sup>+/+Arx<sup>-/-</sup></sup> and Gcg<sup>+/+Arx<sup>PL/PL</sup></sup>, were then mated with male Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice to obtain 6 different male Gcg<sup>+/+Arx<sup>-/-</sup></sup>, Gcg<sup>+/+Arx<sup>PL/PL</sup></sup>, Gcg<sup>+/+Arx<sup>-/-</sup></sup>, Gcg<sup>+/+Arx<sup>-PL/PL</sup></sup>, and Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice. All mice were housed in specific pathogen-free (SPF) barrier facilities in the Research Institute of Environmental Medicine, Nagoya University, and maintained on a 12-h light, 12-h dark cycle and constant temperature (23 °C) with free access to certified chow (Lab Animal Diet MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and distilled water. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The protocol was approved by the Institutional Animal Care and Use Committee of Research Institute of Environmental Medicine, Nagoya University (Permit numbers: #12114). All efforts were made to minimize suffering of animals.

Genotyping

DNA was extracted from cells or tail tips by proteinase K digestion and phenol/chloroform extraction. Genotypes were determined by PCR as previously described in detail [4,10].

Measurement of blood glucose levels and insulin tolerance test (ITT)

Blood samples were obtained from neck blood vessels and from tail veins in two weeks old and four months old male mice, respectively. Glucose levels were determined using a Medisafe glucometer (TERUMO, Tokyo, Japan). For the ITT, mice were starved for 4 h and then injected intraperitoneally with porcine insulin (0.25 units/kg, Sigma-Aldrich Japan, Tokyo, Japan). Tail blood samples were taken at 0, 30, 60, 90 and 120 minutes after the injection and blood glucose levels were determined.

Fluorescent imaging

Two-week-old mice were killed by cervical dislocation. They were immediately dissected to expose the viscera including the pancreas. Fluorescence images, with exposure for 1 or 10 seconds, were captured using a VB-G25 epifluorescence microscope system (Keyence Corp., Osaka, Japan).

RNA extraction and analysis of gene expression

Total RNA was extracted from the pancreas using a RNeasy mini kit (QIAGEN, Germantown, MD) according to the manufacturer’s instructions and cDNAs (cDNAs) were synthesized using random primers. cDNA aliquots equivalent to 1 μg of total RNA were subjected to quantitative real-time PCR. The sequences of the primers used for the analyses are available upon request. Details of the procedure have been described previously [12].

Histological and quantitative analyses on the pancreatic islets

Each pancreas was dissected, weighed, fixed in 4% paraformaldehyde, and then, embedded in paraffin. The islet area was determined using slight modifications of the previously described method [13,14,15]. In brief, the complete pancreas in the paraffin block was cut into 6 μm sections. Sections at 90 μm intervals were stained with hematoxylin and eosin (HE staining) and images of the pancreas were obtained and the dimension/area of the islets was measured using a Nanozoomer 2.0 RS whole slide scanner (Hamamatsu Photonics, Hamamatsu City, Japan). The total pancreatic area was measured using Image Pro Plus 6.1 software (Media Cybernetics, Silver Spring, MD). Islet area was expressed as a proportion (%) of total of the pancreatic area and the density of islets was expressed as per mm<sup>2</sup>. Representative images of HE stained sections were captured using an Olympus BX55 system (Olympus Corporation, Tokyo, Japan).

Antibodies

The anti-glucagon and insulin antibodies were purchased from Abcam, plc., (Cambridge, UK) and the anti-GFP from Medical & Biological Laboratories Co., LTD. (Nagoya, Japan). The HRP-conjugated secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

Statistical analyses

Data are expressed as the mean ± SEM. Statistical analysis was performed using a one-way ANOVA followed by Scheffe’s test, using IBM SPSS Statistics software Version 16.0. P-values less than 0.05 were regarded as statistically significant.

Results

Ontogenetic expression of transcription factors in the pancreas of Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice

The levels of expression of several transcription factors involved in the differentiation of islet endocrine cells were previously shown to be significantly increased in Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice, which develop hyperplasia of GFP-positive α-like cells [3]. To characterize the onset of this altered gene expression pattern, we quantified the levels of transcription factor mRNAs during ontogenesis. As shown in Fig. 1A, significantly higher levels of Arx mRNA were present at postnatal day 3 (P3) in the Gcg<sup>+/+Arx<sup>-/-</sup></sup> pancreas compared to either Gcg<sup>+/+Arx<sup>-/-</sup></sup> or Gcg<sup>+/+Arx<sup>-/-</sup></sup>. By contrast, similar inter-genotype differences in the levels of MafB, Isl-1, and Pax6 mRNAs were not observed until P7 or later. Therefore, the higher level of expression of Arx precedes that of the other transcription factor genes analyzed here. This finding suggests that ARX plays a key role in the development of hyperplasia of α-like cells in the Gcg<sup>+/+Arx<sup>-/-</sup></sup> pancreas.

Body weights and blood glucose levels in Glucagon/Arx double mutant mice

To address the role of ARX in the hyperplasia of α-like cells, we generated Glucagon/Arx (Gcg/Arx) double mutant mice. We crossed Gcg<sup>+/+Arx<sup>-/-</sup></sup> males with females heterozygous for the Arx mutation and obtained Gcg/Arx double mutant mice in the expected Mendelian ratio. At 2 weeks of age, the body weights of Arx<sup>-/-</sup> mice, combined with either Gcg<sup>+/+Arx<sup>-/-</sup></sup> or Gcg<sup>+/+Arx<sup>-/-</sup></sup> were significantly smaller than control Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice (Table 1). The difference in body weight between Gcg<sup>+/+Arx<sup>-/-</sup></sup> and Gcg<sup>+/+Arx<sup>-/-</sup></sup> mice was also
statistically significant. However, the body weights of Gcggfp/+ArxPL/Y mice were comparable to the control. Blood glucose levels in Gcggfp/gfpArx7/Y mice were lower than in Gcggfp/gfpArxX/Y mice. These findings indicate that the functional impairment associated with ARX-7 is more severe than for ARX-PL; this conclusion is in concordance with the difference in severity of neurological phenotypes between these Arx mutant mice [10]. Furthermore, most of the Arx7/Y mice died within 3 months, while ArxPL/Y mice survived for more than 6 months. At 4 months of age, no significant differences in blood glucose levels were detected among Gcggfp/+Arx+/Y, Gcggfp/+ArxPL/Y, and Gcggfp/+ArxPL3 mice. On insulin loading, the changes in blood glucose levels in the Gcggfp/+ArxPL/Y mice were comparable to those in Gcggfp/+Arx+/Y mice (Fig. 2). Taken together, these findings indicate that impairment in growth and blood glucose level control is marginal in the ArxPL/Y mice, but is more severe in the Arx7/Y mice.

Expression of mRNAs for Gfp, glucagon, insulin and Arx in the pancreas of Gcg/Arx double mutant mice

In Gcg/+ mice, hyperplasia of GFP-positive β-like cells results in an increased level of Gfp mRNA and an absence of glucagon mRNA [3]. To address the effect of impaired ARX function on the pancreas, we analyzed expression of Gfp, glucagon, insulin and Arx in the Gcg/Arx double mutant mice.

As shown in Fig. 3A, the levels of Gfp mRNA were significantly higher in the pancreas of Gcg/+Arx+/+ mice than in Gcg/+Arx+/− mice; Gfp levels were significantly lower in Gcg/+Arx+/−, Gcg/+Arx/− and Gcg/−Arx+/− mice than in Gcg/+Arx+/+ mice. This result indicated that Gfp mRNA expression and/or hyperplasia of β-like cells are reduced in the Arx mutant mice. Glucagon mRNA was detected in Gcg/+Arx+/− mice, but not Gcg/+Arx−/− mice, and its expression was also significantly lower in Arx mutant mice (Fig. 3B). As expected from the difference in functional impairment of ARX-PL and ARX-7, the decreases in Gfp and glucagon mRNAs were more evident in Arx+/− mice than in Arx+/+ mice. The levels of insulin mRNA did not differ significantly among the Gcg/Arx double mutants, suggesting that β-cell mass and/or gene expression in β-cells are marginally affected by Arx mutation and by presence or absence of proglucagon-derived peptides (Fig. 3C). The level of Arx mRNA was also increased in the Gcg/+ mice, and was also lower in Arx mutant mice (Fig. 3D).

Islet area, islet number and pancreas size in the Gcg/Arx double mutant mice

The number of islets and the area they encompass are significantly increased in the Gcg/+ mice [3,15]. To address...
whether functionally defective mutation of ARX influence islet number and area, we performed a histological analysis of the pancreas of \textit{Gcg/Arx} double mutant mice at 2 weeks of age. As shown in the representative section Fig. 4A, islet area in the \textit{Gcggfp/Arx} pancreas was greater than in the \textit{Gcggfp/Arx} pancreas. Morphometric analyses confirmed that both islet area (Fig. 4B) and islet number (Fig. 4C) were increased in \textit{Gcggfp/Arx} mice. However, the increase was significantly lower in \textit{Gcggfp/ArxPL} and \textit{Gcggfp/Arx7} mice. These results indicate that ARX is

| Table 1. Phenotype of the mutant mice. |
|----------------------------------------|
| \textbf{Life span} | \textbf{Gcggfp/Arx}^{+/Y} | \textbf{Gcggfp/Arx}^{+/Y} | \textbf{Gcggfp/Arx}^{+/Y} | \textbf{Gcggfp/Arx}^{+/Y} | \textbf{Gcggfp/Arx}^{+/Y} | \textbf{Gcggfp/Arx}^{+/Y} |
|-------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Body Weight (2w old) (n = 5) | 6.56 ± 0.45 ({\textit{d}, ee, ff}) | 5.75 ± 0.90 (f) | 6.27 ± 0.75 (m) | 5.19 ± 0.40 | 5.17 ± 0.32 | 4.46 ± 0.21 |
| Body Weight (4m old) (n = 5) | 28.73 ± 0.64 | 29.12 ± 1.49 | 30.0 ± 2.0 | 27.71 ± 1.42 | No data | No data |
| Blood Glucose (mg/dl) (n = 5) | 107 ± 29 | 105 ± 50 | 107 ± 25 | 66 ± 19 | 90 ± 16 | 45 ± 15 |

\textit{d}: vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.05. \textit{ee}: vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01. \textit{f}: vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.05. \textit{m}: vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01. 

**Figure 3. Expression of \textit{Gfp}, \textit{glucagon}, \textit{insulin} and \textit{Arx} mRNAs in the pancreas of \textit{Gcg/Arx} double mutant mice.** Relative mRNA levels for \textit{Gfp} (A) \textit{glucagon} (B), \textit{insulin1} (C), and \textit{Arx} (D) in the pancreas of 2 weeks old mice are shown. Number of animals is indicated in parenthesis in each column. ‘aa’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01; ‘bb’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01; ‘cc’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01; ‘dd’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01; ‘ee’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01; ‘ff’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.05; ‘ff’ vs \textit{Gcggfp/Arx}^{+/Y}, \textit{P}, 0.01.

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involved in the increase in islet number and area caused by the absence of proglucagon-derived peptides.

An increase in pancreas size/weight has been documented in animal models with defects in glucagon action [3,16], although the mechanism remains unclear. In the present study, we found no evidence of a significant difference in relative pancreas weights among Gcggfp/+Arx+/Y, Gcggfp/+ArxPL/Y and Gcggfp/+Arx7/Y mice. The pancreas weight of Gcggfp/+ArxPL/Y and Gcggfp/+Arx7/Y mice was significantly larger than in Gcggfp/+ mice (Fig. 4D). However, Gcggfp/+Arx+/Y and Gcggfp/+Arx7/Y mice showed a significant difference in pancreas weight at 2 weeks of age, and Gcggfp/+Arx+/Y and Gcggfp/+ArxPL/Y were significantly different at 3 months of age (Fig. 4E). Three-month-old Gcggfp/+Arx7/Y mice were not available for this analysis because of their shortened life span. Overall, the results suggest that ARX is also involved in the increase in pancreas weight caused by the absence of proglucagon-derived peptides. However, the impact of functionally defective ARX on pancreas weight is not as dramatic as that on islet endocrine cells.

**Immunohistochemical analyses and fluorescent imaging of the Gcg/Arx double mutant pancreas**

To characterize the distribution of α/β-like cells and β-cells in the islets, serial sections of the pancreas were immunohistochemically analyzed (Fig. 5A). Immunoreactivity for GFP was markedly increased in the Gcg/dplArx+/Y mice compared to Gcg/dplArx+/Y mice (Fig. 5A, a vs d) indicating marked hyperplasia of α-like cells. There is less apparent hyperplasia of α-like cells in the Gcg/dplArxPL/Y mice (Fig. 5A) and the immunoreactivity was almost comparable to that in Gcg/dplArx+/Y mice (Fig. 5Aa). It was difficult to detect α-cells in Gcg/dplArx+/Y mice and α-like cells in Gcg/dplArx7/Y mice. Glucagon immunoreactivity was present in the Gcg/dpl mice (Fig. 5Ab, h and n), but not in the Gcg/dpl mice (Fig. 5Ac, k and q), and was difficult to detect in Gcg/dplArx7/Y mice (Fig. 5An). By contrast, immunoreactivity for insulin showed little variation between the different genotypes (Fig. 5Ac, f, i, j, m and r).

As α/β-like cells express GFP in the Gcg/Arx double mutant mice, we carried out an in situ analysis of the pancreas using an epifluorescent microscope to identify and estimate the numbers of these cells (Fig. 5B). The results were in agreement with those above for the immunohistochemical analyses (Fig. 5A) and the gene expression data (Fig. 2). Taken together, hyperplasia of α-like cells in the absence of proglucagon-derived peptides was reduced in mice with functionally defective ARX. The number of α-cells was also reduced in Gcg/dpl mice with functionally defective ARX. The present study on the pancreas and pancreatic islets demonstrated that the ARX-7 mutation has a greater effect than ARX-PL, a conclusion that is in agreement with the results of neurological analyses [10].

**Discussion**

Hyperplasia of α-cells has been documented in various mouse models that have defective glucagon action, such as deficient in glucagon receptor [16] or in prohormone convertase 2 that excise glucagon from its precursor proglucagon [17]. Recently, hyperplasia of α-cells has been also shown in mice with a liver-specific knock out of glucagon receptor, suggesting that circulating factors other than glucagon itself regulate α-cell proliferation [18].

The Gcg/dpl mice lack proglucagon-derived peptides, including glucagon and GLP-1, and, as adults, they are normoglycemic. In these aspects, the Gcg/dpl mice are a contrast to with the animal models mentioned above, which exhibit lower blood glucose levels and elevated GLP-1 levels. Nevertheless, the Gcg/dpl mice develop hyperplasia of GFP-positive α-like cells and this result indicates that neither lower blood glucose levels nor elevated GLP-1 levels are prerequisite for the hyperplasia [3,4]. This conclusion is in agreement with a recent report on glucagon receptor/GLP-1
receptor double knockout mice, which are normoglycemic and develop \( \alpha \)-cells hyperplasia [19].

As the underlying mechanisms for hyperplasia of \( \alpha/\beta \)-like cells under defective glucagon action remain largely unknown, we sought to characterize the possible role of ARX in hyperplasia in the present study. We showed that hyperplasia of \( \alpha \)-like cells due to absence of the proglucagon-derived peptides was reduced in male mice lacking a fully functional ARX. In particular, the number of \( \alpha \)-cells in the \( \text{Gcgff} \text{p}/+\text{Arx}^+/Y \) background and \( \alpha \)-like cells in the \( \text{Gcgff} \text{p}/\text{gfp} \text{Arx}^{+/Y} \) background were considerably lower in the mice which carried only ARX-7. The clinical severity of the condition shown by human patients carrying mutations corresponding to those here and the neurological studies of animal models, demonstrates that functional impairment of ARX-7 is more severe than that of ARX-PL [10]. Therefore, our data indicated that the function of ARX is one of the most important modifiers of the number of islet \( \alpha/\beta \)-like cells.

The present study by itself cannot exclude the possibility that the islet phenotype is secondary to neurological disorder caused by defective ARX function. However, as \( \alpha \)-cell specific ablation of ARX causes extensive loss of \( \alpha \)-cells [20], it is likely that functional impairment of ARX in the islet endocrine cells plays the major role in the islet phenotype observed in the present study.

In addition to the increase in islet mass, the size of the pancreas was increased in \( \text{Gcgff} \text{p}/\text{gfp} \text{Arx}^{+/Y} \) mice [4] and in glucagon receptor deficient mice [16]. The effect on pancreas size was also found to be diminished in the present study (Fig. 4D and E), suggesting that ARX is involved in growth of exocrine glands. Interestingly, both loss of \( \alpha \)-cells and morphological abnormalities in exocrine glands have been documented in human patients with Arx-null mutation [21]. As ARX is not expressed in exocrine glands, it is suggested that ARX-dependent signals, i.e. growth factors, from the endocrine pancreas control development and/or maintenance of the exocrine pancreas.

Hyperplasia of \( \alpha \)-cells under defective glucagon signaling is not unique to rodent models, and a human case carrying a homozygous glucagon receptor mutation has been reported to show \( \alpha \)-cell hyperplasia and islet cell tumor [22]. Furthermore,
proliferation of α-cells and disregulated glucagon production have been recently highlighted in the pathogenesis of diabetes mellitus [23,24]. The present study showed that ARX plays an important role in the control of α-cell numbers, and suggests that modification of ARX function and/or expression in islets should be considered as a possible target for suppression of α-cell proliferation.

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Author Contributions

Conceived and designed the experiments: YH YM. Performed the experiments: SX MI YT YH. Analyzed the data: SX YT YH YM. Wrote the paper: SX YM.

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