主論文の要約

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

（転写因子 ARX (Aristaless-related homeobox)はプログルカゴン遺伝子由来ペプチド欠損マウスにおける膵島α細胞の過形成に不可欠な役割を果たす）

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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*). In order to gain insights into the physiological function of proglucagon-derived peptides, we generated *Gcg*-GFP (green fluorescent protein) knock-in mice ($Gcg^{gfp/+}$). In homozygous $Gcg^{gfp/gfp}$ mice lacking all proglucagon-derived peptides, hyperplasia of islet α-like cells which were GFP-positive but could not secrete glucagon were observed. We also found that the hyperplasia of α-like cells was associated with marked increase in expression of mRNA for a transcription factor, Aristaless-related homeobox (ARX), which was originally identified as an important factor for brain development. Since ARX is also known to play a pivotal role in the development of pancreatic α-cells, we aimed to characterize the role of ARX in the hyperplasia of GFP-positive, α-like cells in the pancreatic islets of $Gcg^{gfp/gfp}$ mice by generating the double mutant mice for *Arx* and proglucagon. Because *Arx* null mice die at 2 days after birth, we obtained two mouse strains with partial defects in ARX functions: one strain has elongation of GCG-triplet repeats (330ins[GCG]7) and the other strain has an amino acid substitution (P355L). Functional impairment of ARX-330ins[GCG]7 is more severe than ARX-P355L, as the $Arx^{[330insGCG]7/Y}$ ($Arx^{7/Y}$) mice exhibit greater neurological abnormalities than $Arx^{P355L/Y}$ ($Arx^{PL/Y}$) mice.

【Materials and Methods】

Male mice with 6 different genotypes ($Gcg^{gfp/+}Arx^{+/Y}$, $Gcg^{gfp/+}Arx^{PL/Y}$, $Gcg^{gfp/+}Arx^{7/Y}$, $Gcg^{gfp}/gfpArx^{+/Y}$,$Gcg^{gfp/gfp}Arx^{PL/Y}$ and $Gcg^{gfp/gfp}Arx^{7/Y}$) were obtained by mating female *Arx* mutant mice (Arx-330ins[GCG]7 and Arx-P355L) with male $Gcg^{gfp/gfp}$ mice followed by backcrossing the F1 female mice to the male $Gcg^{gfp/gfp}$ mice. The protocol was approved by the Institutional Animal Care and Use Committee of Research Institute of Environmental Medicine, Nagoya University (Permit number: #12114).

Total RNA was extracted from the pancreas and mRNAs were determined by quantitative real-time PCR. For histological studies with quantitative analyses on the pancreatic islets, the complete pancreas in the paraffin block was cut into 6 μm sections. Sections at 90 mm 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. The total pancreatic area was measured using Image Pro Plus 6.1 software.

Data are expressed as the mean±SEM, and statistical analysis was performed using one-way ANOVA followed by Scheffe's test. *P*-values less than 0.05 were regarded as statistically significant.

【Results】

Ontogenetic expression of transcription factors in the pancreas of *Gcg*$^{gfp/gfp}$ mice

As shown in Fig.1A, significantly higher levels of *Arx* mRNA were present at postnatal day 3 (P3) in the *Gcg*$^{gfp/gfp}$ pancreas compared to either *Gcg*$^{+/+}$ or *Gcg*$^{gfp/+}$. Since similar inter-genotype differences in the levels of *MafB*, *Isl-1* and *Pax6* mRNAs were not observed until P7 or later (Fig.1B, C, D), it is indicated that the higher level of expression of *Arx* precedes that of the other transcription factor genes analyzed here.

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

The body weights of *Arx*$^{7/Y}$, combined with *Gcg*$^{gfp/+}$ or *Gcg*$^{gfp/gfp}$ were significantly smaller than the control (*Gcg*$^{gfp/+}$*Arx*$^{+/Y}$) mice whereas the body weights of *Gcg*$^{gfp/+}$*Arx*$^{PL/Y}$ mice were comparable to the control (Table1). Blood glucose levels in *Gcg*$^{gfp/gfp}$*Arx*$^{7/Y}$ mice were lower than in *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ mice. On insulin loading, the changes in blood glucose levels in the *Gcg*$^{gfp/+}$*Arx*$^{PL/Y}$ mice were comparable to those in *Gcg*$^{gfp/+}$*Arx*$^{+/Y}$ mice (Fig.2). Taken together, these findings indicate that impairment in growth and blood glucose level control is marginal in the *Arx*$^{PL/Y}$ mice, but is more severe in the *Arx*$^{7/Y}$ mice.

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

The levels of *Gfp* mRNA were significantly higher in pancreas of *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ mice than *Gcg*$^{gfp/+}$*Arx*$^{+/Y}$ mice; *Gfp* levels were significantly lower in *Gcg*$^{gfp/gfp}$*Arx*$^{PL/Y}$ and *Gcg*$^{gfp/gfp}$*Arx*$^{7/Y}$ mice than in *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ mice (Fig.3A). *Glucagon* mRNA was detected in *Gcg*$^{gfp/+}$ and its expression was also lower in *Arx* mutant mice (Fig.3B). The levels of *insulin* mRNA were no change among these mice (Fig.3C). The level of *Arx* mRNA was increased in *Gcg*$^{gfp/gfp}$ 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

As shown in Fig. 4A, islet area in the *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ pancreas was greater than in the *Gcg*$^{gfp/+}$*Arx*$^{+/Y}$ pancreas. Morphometric analyses confirmed that both islet area (Fig.4B) and islet number (Fig.4C) were increased in *Gcg*$^{gfp/gfp}$ mice whereas the increase was significantly lower in *Gcg*$^{gfp/gfp}$*Arx*$^{PL/Y}$ and *Gcg*$^{gfp/gfp}$*Arx*$^{7/Y}$ mice. The pancreas weight of *Gcg*$^{gfp/gfp}$*Arx*$^{PL/Y}$ and *Gcg*$^{gfp/gfp}$*Arx*$^{7/Y}$ mice was significantly larger than in *Gcg*$^{gfp/+}$mice (Fig. 4D). However, *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ and *Gcg*$^{gfp/gfp}$*Arx*$^{7/Y}$ mice showed a significant difference in pancreas weight at 2 weeks of age, and *Gcg*$^{gfp/gfp}$*Arx*$^{+/Y}$ and *Gcg*$^{gfp/gfp}$*Arx*$^{PL/Y}$ were also significantly different at 3 months of age (Fig. 4E).

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

Immunoreactivity for GFP was markedly increased in the $Gcg^{gfp/gfp}Arx^{+/Y}$ mice compared to $Gcg^{gfp/+}Arx^{+/Y}$mice (Fig. 5A, a vs d). There is less apparent hyperplasia of α-like cells in the $Gcg^{gfp/gfp}Arx^{PL/Y}$ mice (Fig. 5A j) and the immunoreactivity was almost comparable to that in $Gcg^{gfp/+}Arx^{+/Y}$ mice (Fig. 5A a). It was difficult to detect α-cells in $Gcg^{gfp/gfp}Arx^{7/Y}$ mice and α-like cells in $Gcg^{gfp/gfp}Arx^{7/Y}$ mice (Fig 5A m and p). Glucagon immunoreactivity was present in the $Gcg^{gfp/+}$ mice (Fig. 5A b, h and n), but not in the $Gcg^{gfp/gfp}$ mice (Fig. 5A e, k and q), and was difficult to detect in $Gcg^{gfp/+}Arx^{7/Y}$ mice (Fig. 5A n). By contrast, immunoreactivity for insulin showed little variation between the different genotypes (Fig. 5A c, f, i, l, o and r). Results of epifluorescent microscope were in agreement with those for the immunohistochemical analyses (Fig. 5B).

【Discussion】

As the underlying mechanisms for hyperplasia of α/α-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 α-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 α-cells in the $Gcg^{gfp/+}$ background and α-like cells in the $Gcg^{gfp/gfp}$ 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.

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

【Conclusion】

From our present results it is indicated that the function of ARX is one of the most important modifiers of the number of islet α/α-like cells and thus plays a key role in hyperplasia of α-like cells in mice deficient in proglucagon-derived peptides.