Biochemical and Biophysical Research Communications **292,** 138–143 (2002)
doi:10.1006/bbrc.2002.6601, available online at http://www.idealibrary.com on IDEAL®

Transgenic Expression of FGF8 and FGF10 Induces Transdifferentiation of Pancreatic Islet Cells into Hepatocytes and Exocrine Cells

Takashi Yamaoka,*[,1] Kenji Yoshino,† Taketo Yamada,‡ Makiko Yano,† Takefumi Matsui,* Takashi Yamaguchi,* Maki Moritani,* Jun-ichi Hata,‡ Sumihare Noji,§ and Mitsuo Itakura*

**Division of Genetic Information, Institute for Genome Research, †Otsuka Department of Molecular Nutrition, School of Medicine, and §Department of Biological Science and Technology, Faculty of Engineering, University of Tokushima, Tokushima 770-8503, Japan; and ‡Department of Pathology, School of Medicine, Keio University, Tokyo 160-0016, Japan*

Received January 30, 2002

FGF signaling is essential for normal development of pancreatic islets. To examine the effects of overexpressed FGF8 and FGF10 on pancreatic development, we generated FGF8- and FGF10-transgenic mice (Tg mice) under the control of the glucagon promoter. In FGF8-Tg mice, hepatocyte-like cells were observed in the periphery of pancreatic islets, but areas of α and β cells did not decrease, whereas in FGF10-Tg mice, pancreatic ductal and acinar cells were found in islets, concomitantly with disturbed β-cell differentiation. These results suggest that FGF8 and FGF10 play important roles in development of hepatocytes and exocrine cells, respectively, and explain the absence of FGF8 expression in normal islets and pancreatic hypoplasia in FGF10-deficient mice.

Key Words: **transgenic mice; fibroblast growth factor 8; fibroblast growth factor 10; pancreatic islet; hepatocyte; transdifferentiation; glucagon promoter; anophthalmia; thyroid cancer.**

Fibroblast growth factor (FGF) signaling is indispensable for normal development of pancreatic islets. In the earliest stage of pancreatic development, FGF2 secreted from notochord represses sonic hedgehog expression in adjacent nascent pancreatic endoderm, and permits expression of pancreatic genes including pancreatic and duodenal homeobox 1 (Pdx1) and insulin (1). FGF1, FGF2, FGF4, FGF5, FGF6, FGF7, and FGF10 (2–5), and FGF receptors (FGFR) 1, 2, 3, 4, and 5 (2, 4, 6–8) are expressed in pancreatic islets. In transgenic mice (Tg mice) expressing a dominant-negative form of FGFR1c under the control of the Pdx1 promoter, the number of β cells decreases, leading to diabetes (2). Soluble FGFR2b dominant negative mutants, which are thought to primarily block FGF10 signaling (9), cause a greatly reduced number of morphologically aberrant acinar cells and no detectable islets in Tg mice (10) and a significant reduction in both DNA content and amylase activity of cultured pancreatic rudiments (11). Indeed, both FGFR2b-null and FGF10-null mice exhibit pancreatic dysgenesis (9, 12).

In vertebrate limb bud, FGF10 induces FGF8 expression (13) and both are essential for normal limb formation (14–16). However, FGF8 expression was not detected in both fetal and adult mouse pancreas even by reverse transcriptase-polymerase chain reaction (RT-PCR), in spite of FGF10 expression. Thus, to clarify the necessity of the absence of FGF8 and the function of FGF10 in pancreatic development, we generated Tg mice expressing FGF8 or FGF10 in pancreatic islets.

Although the insulin promoter has been used for islet-specific transgene expression, the possibility has been suggested that pancreatic β cells are nonspecifically destroyed by the transgenic overexpression of various proteins driven by the insulin promoter (17). To circumvent this issue, we used the glucagon promoter for the islet-specific expression of FGF8 and FGF10 in Tg mice.

MATERIALS AND METHODS

Generation of Tg mice. The expression vector including the rat glucagon promoter (0.9 kb) for Tg mice was constructed previously (18), and mouse FGF8 cDNA or FGF10 cDNA (a gift from Dr. Katsumata, Sumitomo Pharmaceuticals Research Center, Osaka, Japan) was inserted into its cloning site flanking the exon-intron organization and a polyadenylation signal of the rabbit β-globin gene. An *Xho*I fragment of this vector excluding plasmid-derived sequences was used as the transgene, and microinjected into the male pronuclei of fertilized eggs obtained from superovulated BDF1 (C57BL/6 ×

[1] To whom correspondence and reprint requests should be addressed. Fax: +81-88-633-9484. E-mail: yamaoka@genome.tokushima-u.ac.jp.

0006-291X/02 $35.00

DBA2 F1) female mice crossed with males of the same strain. Injected embryos were implanted into the oviducts of pseudopregnant female mice and allowed to develop (19). DNA was extracted from tail snips of live offspring by the proteinase K/SDS method. The integration and copy number of the transgene in the mouse genome were examined by Southern blot analysis or PCR. F1 transgenic progeny was bred by crossing Tg founder mice with BDF1 mice, and F1 Tg mice of 10 to 30 weeks of age were used with sex-matched nontransgenic littermates (wild-type mice) in this study. All mice were handled according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised 1985).

Gene expression in pancreatic islets. From mouse islets collected by the collagenase method (20), total RNA was extracted with ISOGEN (Nippon Gene, Tokyo, Japan). Semiquantitative RT-PCR was performed as previously described (21) and each gene expression level was standardized by the β-actin expression level. Used sense and antisense primers are respectively as follows: 5′-CGTGCTGGAGAACAACTACA-3′ and 5′-GGGTAGTTGAGGAACTCGAAG-3′ for mouse FGF8, 5′-TTGGTGTCTTCGTTCCCTGT-3′ and 5′-CATTTGCCTGCCATTGTGCT-3′ for mouse FGF10, 5′-ATCCTGAGAACTTCAGGCTC-3′ and 5′-AATGATGAGACAGCACAACA-3′ for transgene, 5′-CTGCATACTGGAGCACTTCA-3′ and 5′-TGCTCATACGATGAGCATGC-3′ for mouse albumin, 5′-AGCCAAGGACAGAATGGCTG-3′ and 5′-AAGACATCAGTGTCTGCTGG-3′ for mouse αFP, and 5′-GTGGGCCGCTCTAGGCACCA-3′ and 5′-CGGTTGGCCTTAGGGTTCAGG-3′ for mouse β-actin.

Histopathological examinations. Mouse pancreas was stained with hematoxylin and eosin (H&E). For immunohistochemistry, guinea-pig polyclonal antibodies against porcine insulin and glucagon (DAKO Japan, Kyoto, Japan) were used. The quantitative analysis of the islet-cell area was carried out as previously described (19). In brief, islet area, α-cell area, and β-cell area were identified by H&E staining, glucagon immunostaining, and insulin immunostaining, respectively.

Statistical analysis. All data are presented as means ± SEM. For comparison of two means, Student's unpaired t test was used. For comparison of two ratios (Q_1, Q_2), a normal distribution curve with a mean of $Q_1 - Q_2$ and a variance of $Q_1(1 - Q_1)/n_1 + Q_2(1 - Q_2)/n_2$ was used. Probability (P) of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Generation of FGF8- and FGF10-Tg Mice

Two FGF8-Tg founder mice and three FGF10-Tg founder mice were obtained. The transgene copy numbers of FGF8-Tg mice of lines 1, 2, and FGF10-Tg mice of lines 1, 2, and 3 were 1, 1, 1, 4, and 4, respectively. The low copy number of 1 in both lines of FGF8-Tg mice suggests that higher copy numbers, i.e., higher FGF8 expression levels, are unfavorable for survival. F1 mice were bred by crossing Tg founder mice with wild-type mice, the percentages of FGF8-Tg mice in F1 mice at the age of 5 weeks were 36% (28/78) in line 1 and 26% (28/107) in line 2, and 30% (56/185) in total, whereas those of FGF10-Tg mice were 45% (31/69) in line 1, 44% (24/55) in line 2, 49% (23/47) in line 3, and 46% (78/171) in total. The percentages of FGF10-Tg mice (46%) are consistent with Mendel's law: 50% of F1 mice are Tg mice, whereas the percentages of FGF8-Tg mice (30%) are significantly ($P < 0.005$) smaller than those of FGF10-Tg mice. Therefore, FGF8-Tg mice are certainly disadvantageous to survival until 5 weeks of age from fetal development.

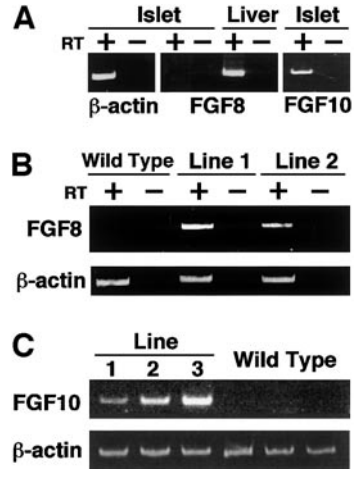

FIG. 1. FGF expression in pancreatic islets. Mice at the age of 10–20 weeks were used. (A) Expression of FGF8 and FGF10 in pancreatic islets of wild-type mice. Mouse FGF8 cDNA in the liver was amplified as a positive control. RT, reverse transcriptase. (B, C) Transgene expression in pancreatic islets of FGF8- and FGF10-Tg mice. Transgene-specific primers were used for PCR. As an internal standard, mouse β-actin cDNA was amplified.

FGF Expression in Pancreatic Islets

In contrast to the relatively high-level expression of FGF8 in mouse liver, FGF8 expression was not detected in pancreatic islets of wild-type mice, while FGF10 was expressed in wild-type islets (Fig. 1A). In all Tg lines, transgene expression was confirmed by semi-quantitative RT-PCR with transgene-specific primers (Fig. 1B,C). In FGF8-Tg mice, transgene expression level in line 1 was higher than that in line 2 (Fig. 1B), and in FGF10-Tg mice, transgene expression level in line 3 was the highest in those of all three lines (Fig. 1C), in association with the highest transgene copy number of 4 in line 3. Transgene expression was not detected in wild-type mice.

Prominent Phenotypes of FGF-Tg Mice

All FGF8-Tg mice exhibited bilateral anophthalmia, although normally developed conjunctivae were remaining (Fig. 2A). Anophthalmia was probably caused by the increase in the blood concentration of FGF8 protein. Although the blood concentration of FGF8 protein could not be determined in this study, a considerable increase in FGF8 protein in blood is speculated for the following reasons: The glucagon promoter, by which transgene was expressed, has a relatively high activity, and starts to function at embryonic day 8.5–9.0 (22), which is the critical period of optic vesicle formation (23). Indeed, in Tg mice expressing interferon-γ (IFN-γ) under the control of this glucagon pro-

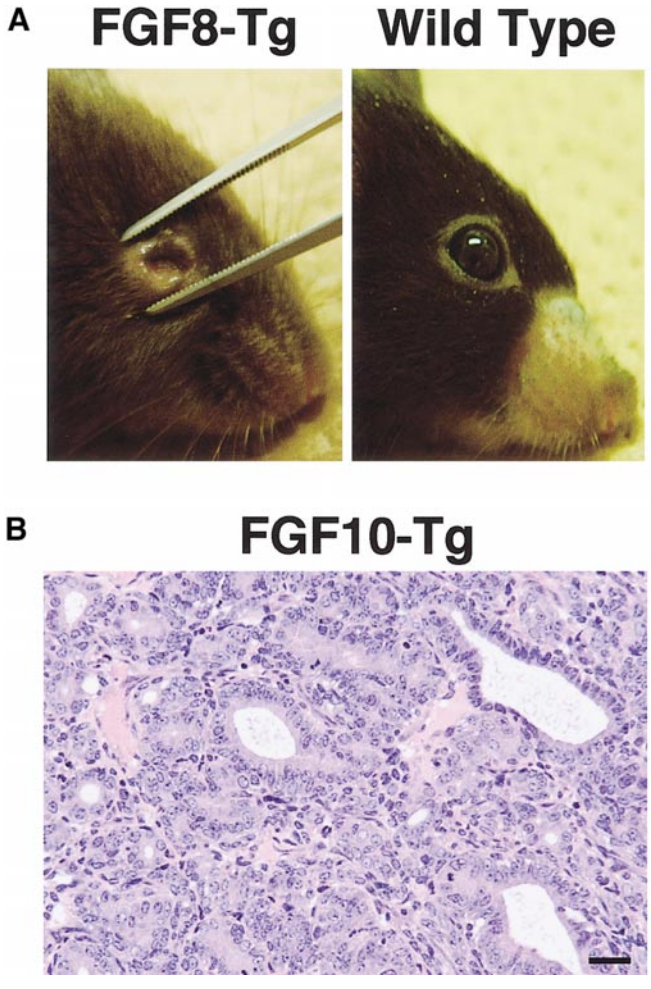

FIG. 2. Conspicuous phenotypes in Tg mice. (A) Anophthalmia in FGF8-Tg mice. All FGF8-Tg mice lacked eyeballs, whereas their conjunctivae developed normally. (B) Thyroid cancer in FGF10-Tg mice (H&E staining). Scale bar, 100 μm.

moter, the average concentration of serum IFN-γ was 50 times higher than that in wild-type mice, and all IFN-γ Tg mice showed microphthalmia (17). Furthermore, FGF8 was reported to play a role in the initiation and differentiation of neural retina and lens (24), and FGF8 expression using the αA-crystallin promoter in Tg mice results in microphthalmia (25). Anophthalmia may explain the lower rate of survival until 5 weeks of age (30%) in our FGF8-Tg mice because complete blindness is likely to be unfavorable for survival including sucking milk.

Various malignant neoplasms developed in FGF10-Tg mice. In total, 18 (39%) out of 46 FGF10-Tg mice were affected until 60 weeks of age: Medullary thyroid cancer (Fig. 2B) was most frequent (8/46; 17%), and followed by neoplasms of lung, liver, testis, and unknown origin (2/46; 4% each), lymphoma (1/46; 2%), and rhabdomyosarcoma (1/46; 2%). FGF10 signaling is essential for thyroid and lung development, i.e., both mice deficient in FGF10 and mice deficient in its receptor (FGFR2b) lack the thyroid gland and lungs (9,12), whereas transgenic overexpression of FGF10 in the lungs causes multifocal pulmonary tumors (26). Therefore, it is suggested that precisely regulated FGF10 expression is necessary for normal tissue development.

Histopathological Examination

In both lines of FGF8-Tg mice, hepatocyte-like cells were observed in the periphery of pancreatic islets (Fig. 3). These cells had large clear nuclei with several

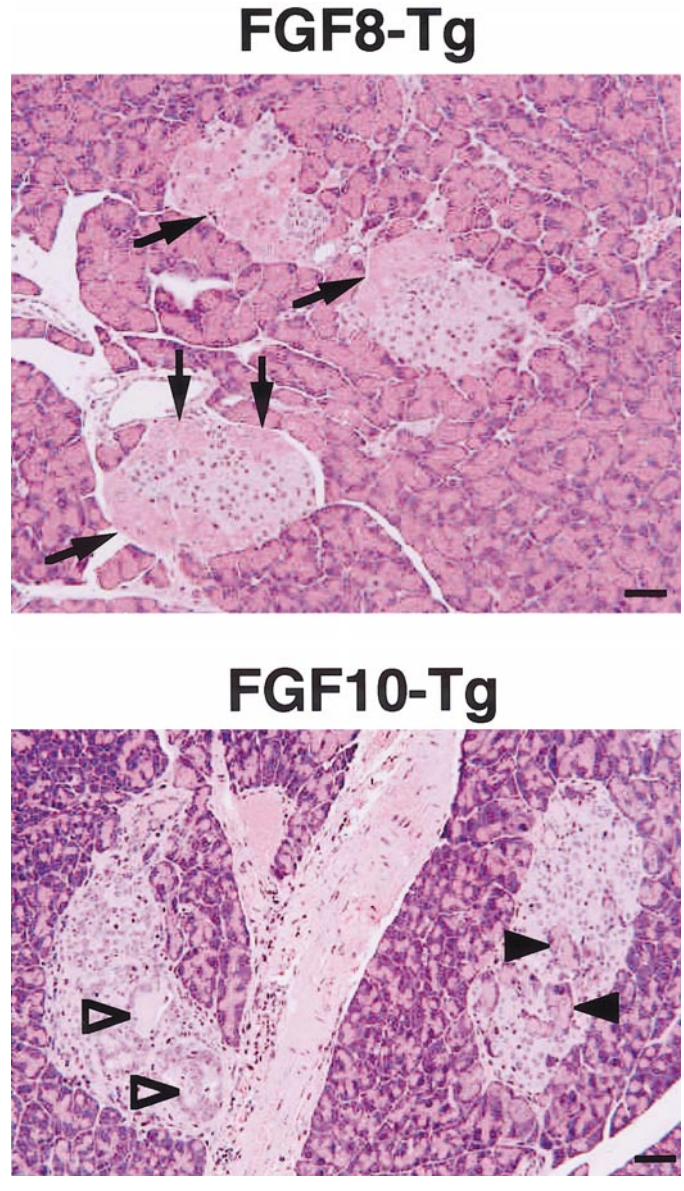

FIG. 3. Transdifferentiation of islet cells. In FGF8-Tg mice, hepatocytes-like cells (arrows) were observed in the periphery of pancreatic islets. In line 3 of FGF10-Tg mice, ductal (white arrowheads) and acinar cells (black arrowheads) were found in pancreatic islets.

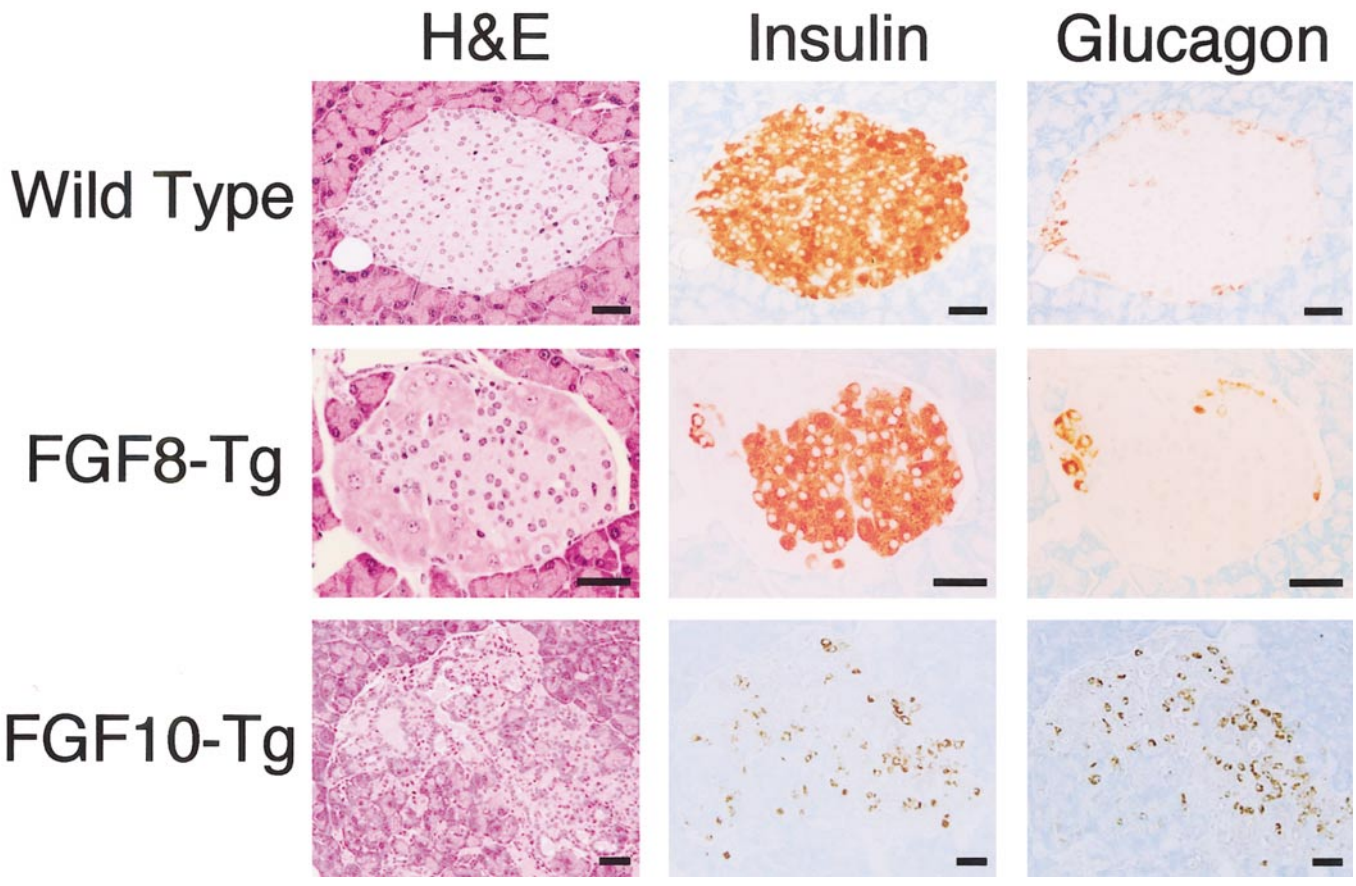

FIG. 4. Immunostaining for insulin and glucagon. Neither insulin nor glucagon was detected in hepatocyte-like cells in FGF8-Tg islets and exocrine cells in FGF10-Tg islets (line 3). In line 3 of FGF10-Tg mice, the numbers of α and β cells were comparable because of the decrease in the number of β cells. H&E, hematoxylin and eosin staining. Scale bars, 100 μm.

small nucleoli and abundant eosinophilic cytoplasm, and were morphologically indistinguishable from hepatocytes. In line 3 of FGF10-Tg mice, ductal and acinar cells were shown in pancreatic islets (Fig. 3), although no changes were found in lines 1 and 2 of FGF10-Tg mice. Because transgene expression level in pancreatic islets of line 3 was several times higher than that of line 1 or 2 (Fig. 1C), FGF10 expression levels in lines 1 and 2 are thought to be lower than the threshold of FGF10 expression level for transdifferentiation of endocrine cells into exocrine cells. Neither hepatocyte-like cells in FGF8-Tg islets nor exocrine cells in FGF10-Tg islets produced insulin or glucagon on the immunohistochemical basis (Fig. 4). Although the ratio of β cells to α cells was similar between wild-type and FGF8-Tg mice, this ratio in line 3 of FGF10-Tg mice was obviously lower because of the decrease in the number of β cells (Fig. 4). Actually, β-cell area in line 3 of FGF10-Tg mice was only 32% that in wild-type mice (Fig. 5), whereas α-cell area in line 3 of FGF10-Tg mice did not decrease. These results indicate that overexpressed FGF10 disturbs normal development of β cells. In FGF8-Tg mice, α- and β-cell areas were respectively similar to those in wild-type mice, suggesting that overexpressed FGF8 does not interfere with the mechanism of maintaining α- and β-cell masses appropriately, although a part of endocrine cells transdifferentiated into hepatocyte-like cells by FGF8. Actually, plasma insulin and glucagon concentrations and pancreatic contents of insulin and glucagon in FGF8-Tg

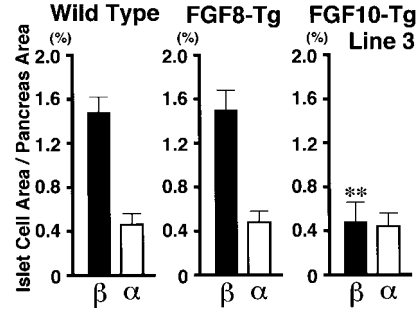

FIG. 5. Morphological examination. Relative areas of α (white bar) and β (black bar) cells to the whole pancreas area were measured. In line 3 of FGF10-Tg mice, β-cell area was about 3 times smaller than that in wild-type mice, whereas α-cell area was similar. In lines 1 and 2 of FGF10-Tg mice and in FGF8-Tg mice, both α- and β-cell areas are respectively comparable to those in wild-type mice. $^{**}P < 0.01$.

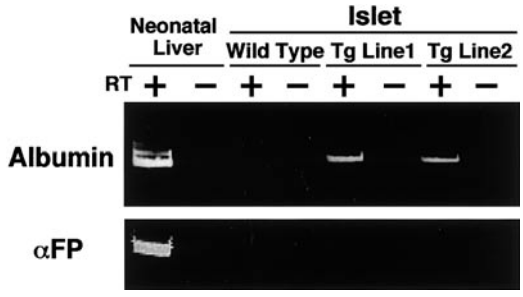

FIG. 6. Liver-specific gene expression. In FGF8-Tg islets, albumin expression was detected by RT-PCR, but αFP expression was not. Neonatal mouse liver was used as a positive control. RT, reverse transcriptase. αFP, α-fetoprotien.

mice are respectively similar to those in wild-type mice (data not shown).

Albumin Expression in FGF8-Tg Islets

Albumin was expressed in FGF8-Tg islets, but α-fetoprotein (αFP) was not (Fig. 6). Because albumin and αFP are the markers for mature and immature hepatocytes, respectively, highly differentiated hepatocytes may be generated in FGF8-Tg islets. Another explanation for the lack of αFP expression is that FGF8 may induce only albumin expression, but not αFP expression, regardless of maturity of hepatocytes.

In the developing mouse embryo, FGF8 is secreted with FGF1 and 2 from cardiac mesoderm, and induces the liver gene expression program and the morphogenetic outgrowth of the hepatic bud in adjacent ventral endoderm (27, 28). Furthermore, bipotential precursor cells for the pancreas and the liver exist within the embryonic ventral endoderm, and FGF signaling diverts the multipotent endoderm cells to a hepatic fate by the induction of sonic hedgehog expression and the suppression of Pdx1 expression (29).

The Origin of Transdifferentiated Islet Cells

In FGF8-Tg mice, the origin of hepatocyte-like cells is unknown. However, these cells are probably derived from endocrine precursor cells because they are in pancreatic islets (Fig. 3). Although they are in the periphery of islets where non-β cells such as α cells are usually located, this finding does not necessarily suggest that hepatocyte-like cells originated from non-β cells. The arrangement of islet cells has been considered to be dependent on adhesion molecules of each islet cell. For example, cadherins are essential for the aggregation of β cells, because in Tg mice expressing a dominant negative mutant of E-cadherin in β cells, the clustering of β cells is disturbed, while α cells selectively aggregate into islet-like clusters without β cells (30). If such adhesion molecules as cadherin are not expressed in hepatocyte-like cells, they lose close contact with β cells and are located outside β-cell aggregate in islets.

In FGF10-Tg mice as well as in FGF8-Tg mice, exocrine cells in pancreatic islets are presumed to be derived from endocrine precursor cells because these exocrine cells are located in islets, but not outside of islets. The endocrine precursor cells was reported to express neurogenin3 without four pancreatic hormones (31, 32), and to be able to differentiate into four types of pancreatic endocrine cells. Recently, multipotential stem cells were found in pancreatic islets (33, 34). These cells produce nestin as well as neural stem cells, and can differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. Therefore, the origin of transdifferentiated cells in FGF8- and FGF10-Tg mice may be nestin-positive stem cells in pancreatic islets.

In conclusion, transgenic expression of FGF8 and 10 induced transdifferentiation of pancreatic islet cells into hepatocyte-like cells and into exocrine cells concomitantly with impaired β-cell differentiation, suggesting that FGF8 and 10 play important roles in development of hepatocytes and pancreatic exocrine cells, respectively. These results explain the absence of FGF8 expression in normal islets and pancreatic hypoplasia in FGF10-deficient mice.

REFERENCES

1. Hebrok, M., Kim, S. K., and Melton, DA. (1998) Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev.* **12,** 1705–1713.
2. Hart, A. W., Baeza, N., Apelqvist, A., and Edlund, H. (2000) Attenuation of FGF signaling in mouse β-cells leads to diabetes. *Nature* **408,** 864–868.
3. Ishiwata, T., Kornmann, M., Beger, H. G., and Korc, M. (1998) Enhanced fibroblast growth factor 5 expression in stromal and exocrine elements of the pancreas in chronic pancreatitis. *Gut* **43,** 134–139.
4. Ishiwata, T., Friess, H., Buchler, M. W., Lopez, M. E., and Korc, M. (1998) Characterization of keratinocyte growth factor and receptor expression in human pancreatic cancer. *Am. J. Pathol.* **153,** 213–222.
5. Arany, E., and Hill, D. J. (2000) Ontogeny of fibroblast growth factors in the early development of the rat endocrine pancreas. *Pediatr. Res.* **48,** 389–403.
6. Hughes, S. E. (1997) Differential expression of the fibroblast growth factor receptor (FGFR) multigene family in normal human adult tissues. *J. Histochem. Cytochem.* **45,** 1005–1019.
7. Oberg-Welsh, C., and Welch, M. (1996) Effects of certain growth factors on *in vitro* maturation of rat fetal islet-like structures. *Pancreas* **12,** 334–339.
8. Kim, I., Moon, S., Yu, K., Kim, U., and Koh, G. Y. (2001) A novel fibroblast growth factor receptor-5 preferentially expressed in the pancreas. *Biochim. Biophys. Acta* **1518,** 152–156.
9. Ohuchi, H., Hori, Y., Yamasaki, M., Harada, H., Sekine, K., Kato, S., and Itoh, N. (2000) FGF10 acts as a major ligand for FGF receptor 2IIIb in mouse multi-organ development. *Biochem. Biophys. Res. Commun.* **277,** 643–649.
10. Celli, G., LaRochelle, W. J., Mackem, S., Sharp, R., and Merlino, G. (1998) Soluble dominant-negative receptor uncovers essential

roles for fibroblast growth factors in multi-organ induction and patterning. *EMBO J.* **17,** 1642–1655.

11. Miralles, F., Czernichow, P., Ozaki, K., Itoh, N., and Scharfmann, R. (1999) Signaling through fibroblast growth factor receptor 2b plays a key role in the development of the exocrine pancreas. *Proc. Natl. Acad. Sci. USA* **96,** 6267–6272.
12. Revest, J. M., Spencer-Dene, B., Kerr, K., De Moerlooze, L., Rosewell, I., and Dickson, C. (2001) Fibroblast growth factor receptor 2-IIIb acts upstream of Shh and Fgf4 and is required for limb bud maintenance but not for the induction of Fgf8, Fgf10, Msx1, or Bmp4. *Dev. Biol.* **231,** 47–62.
13. Ohuchi, H., Nakagawa, T., Yamamoto, A., Araga, A., Ohata, T., Ishimaru, Y., Yoshioka, H., Kuwana, T., Nohno, T., Yamasaki, M., Itoh, N., and Noji, S. (1997) The mesenchymal factor, FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. *Development* **124,** 2235–2244.
14. Lewandoski, M., Sun, X., and Martin, G. R. (2000) Fgf8 signaling from the AER is essential for normal limb development. *Nat. Genet.* **26,** 460–463.
15. Moon, A. M., and Capecchi, M. R. (2000) Fgf8 is required for outgrowth and patterning of the limbs. *Nat. Genet.* **26,** 455–459.
16. Sekine, K., Ohuchi, H., Fujiwara, M., Yamasaki, M., Yoshizawa, T., Sato, T., Yagishita, N., Matsui, D., Koga, Y., Itoh, N., and Kato, S., (1999) Fgf10 is essential for limb and lung formation. *Nat. Genet.* **21,** 138–141.
17. Yamaoka, T., Yano, M., Idehara, C., Yamada, T., Tomonari, S., Morintani, M., Ii, S., Yoshimoto, K., Hata, J., and Itakura, M. (1999) Apoptosis and remodelling of beta cells by paracrine interferon-γ without insulitis in transgenic mice. *Diabetologia* **42,** 566–573
18. Moritani, M., Yoshimoto, K., Tashiro, F., Hashimoto, C., Miyazaki, J., Ii, S., Kudo, E., Iwahana, H., Hayashi, Y., Sano, T., and Itakura, M. (1994) Transgenic expression of IL-10 in pancreatic islet A cells accelerates autoimmune insulitis and diabetes in non-obese diabetic mice. *Int. Immunol.* **6,** 1927–1936.
19. Yamaoka, T., Idehara, C., Yano, M.., Matsushita, T., Yamada, T., Ii, S., Moritani, M., Hata, J., Sugino, H., Noji, S., and Itakura, M. (1998) Hypoplasia of pancreatic islets in transgenic mice expressing activin receptor mutants. *J. Clin. Invest.* **102,** 294–301.
20. Moritani, M., Yoshimoto, K., Ii, S., Kondo, M., Iwahana, H., Yamaoka, T., Sano, T., Nakano, N., Kikutani, H., and Itakura, M. (1996) Prevention of adoptively transferred diabetes in nonobese diabetic mice with IL-10-transduced islet-specific Th1 lymphocytes. *J. Clin. Invest.* **98,** 1851–1859.
21. Yamaoka, T., Yano, M., Yamada, T., Matsushita, T., Moritani, M, Ii, S., Yoshimoto, K., Hata, J., and Itakura, M. (2000) Diabetes and pancreatic tumours in transgenic mice expressing Pax6. *Diabetologia* **43,** 332–339.
22. Gittes, G. K., and Rutter, W. J. (1992) Onset of cell-specific gene expression in the developing mouse pancreas. *Proc. Natl. Acad. Sci. USA* **89,** 1128–1132.
23. Matthew, H. K. (1992) Assessment of developmental stage of pre- and postimplantation mouse embryos based on the staging system of Theiler (1989). *In* The Atlas of Mouse Development, pp. 64–74, Academic Press, San Diego, CA.
24. Vogel-Hopker, A., Momose, T., Rohrer, H., Yasuda, K., Ishihara, L., and Rapaport, D. H. (2000) Multiple functions of fibroblast growth factor-8 (FGF-8) in chick eye development. *Mech. Dev.* **94,** 25–36.
25. Lovicu, F. J., and Overbeek, P. A. (1998) Overlapping effects of different members of the FGF family on lens fiber differentiation in transgenic mice. *Development* **125,** 3365–3377.
26. Clark, J. C., Tichelaar, J. W., Wert, S. E., Itoh, N., Perl, A. K., Stahlman, M. T., and Whitsett, J. A. (2001) FGF-10 disrupts lung morphogenesis and causes pulmonary adenomas *in vivo*. *Am. J. Physiol.* **280,** L705–L715.
27. Jung, J., Zheng, M., Goldfarb, M., and Zaret K. S. (1999) Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* **284,** 1998–2003.
28. Zaret, S. K. (2000) Liver specification and early morphogenesis. *Mech. Dev.* **92,** 83–88.
29. Deutsch, G., Jung, J., Zheng, M., Lora, J., and Zaret, K. S. (2001) A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* **128,** 871–881.
30. Dahl, U., Sjodin, A., and Semb, H. (1996) Cadherins regulate aggregation of pancreatic β-cells *in vivo*. *Development* **122,** 2895–2902.
31. Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabe de Angelis, M., Lendahl, U., and Edlund, H. (1999) Notch-signaling controls pancreatic cell differentiation. *Nature* **400,** 877–881.
32. Gradwohl, G., Dierich, A., LeMeur, M., and Guillemot, F. (2000) *neurogenin3* is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl. Acad. Sci. USA* **97,** 1607–1611.
33. Hunziker, E., and Stein, M. (2000) Nestin-expressing cells in the pancreatic islets of Langerhans. *Biochem. Biophys. Res. Commun.* **271,** 116–119.
34. Zulewski, H., Abraham, E. J., Gerlach, M. J., Daniel, P. B., Moritz, W., Muller, B., Vallejo, M., Thomas, M. K., and Habener, J. F. (2001) Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate *ex vivo* into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* **50,** 521–533.