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Gastric PDX-1 expression in pancreatic metaplasia and endocrine cell hyperplasia in atrophic corpus gastritis

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The homeodomain transcription factor PDX-1 plays a key role in endocrine and exocrine differentiation processes of the pancreas. PDX-1 is also essential for differentiation of endocrine cells in the gastric antrum. The role of PDX-1 in the pathogenesis of endocrine cell hyperplasia and pancreatic metaplasia in corpus and fundus gastritis has not been evaluated. By immunohistochemistry and double-immunofluorescence, we investigated the expression of PDX-1 in 10 tissue specimens with normal human gastric mucosa, nonatrophic and atrophic gastritis and in pancreatic metaplasia, respectively. In normal corpus mucosa and in nonatrophic corpus gastritis, PDX-1 was mainly absent. In pancreatic metaplasia, PDX-1 was found in metaplastic cells and in adjacent gastric glands. In contrast to normal gastric corpus mucosa, PDX-1 could be strongly detected in the cytoplasm of the parietal cells surrounding metaplastic areas. Furthermore, PDX-1 expression was found in hyperplastic endocrine cells and in the surrounding gastric glands in chronic atrophic gastritis. Hyperplastic endocrine cells coexpressed the β-subunit of the gastric H,K-ATPase. We conclude that PDX-1 represents a candidate switch factor for glandular exocrine and endocrine transdifferentiation in chronic gastritis and that an impaired parietal cell differentiation might play a key role in disturbed gastric morphogenic processes.
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In recent years, several factors have been characterized that play a key role in pancreatic organogenesis.[1] The homeobox transcription factor PDX-1 (pancreatic duodenal homeobox-1) also termed IPF-1 could be regarded as master switch for glandular exocrine and endocrine differentiation in the pancreas. Mutations within the *PDX-1* gene are associated with type II diabetes mellitus or even with pancreatic agenesis.[2] In the stomach, PDX-1 has been shown to be essential for the differentiation of endocrine cells in the antropyloric region of mice and targeted deletion of PDX-1 leads to severely impaired development of gastrin cells in the antrum.[3] However, no study concerning the potential role of PDX-1 in glandular differentiation processes of the oxyntic mucosa in the corpus and fundus region has been undertaken. In these areas, pancreatic (acinar) metaplasia is frequently found and is associated with chronic gastritis.[4,5] Histologically, this type of metaplasia is characterized by acinus-like nests of epithelial cells producing pancreatic enzymes such as amylase, lipase and trypsinogen.[4] Furthermore, the so-called hyperplastic endocrine nodules can be regularly found in severe atrophic corpus gastritis. These nodules represent precursor lesions for neuroendocrine tumors within the gastric mucosa.[6] The molecular pathogenesis of both, pancreatic metaplasia and endocrine cell hyperplasia, is only poorly understood. To investigate the potential role of PDX-1 in these pathological conditions within the glandular compartment of the gastric mucosa, we analyzed the PDX-1 expression in normal gastric mucosa, in chronic gastritis with and without body mucosa atrophy and in pancreatic metaplasia.

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Materials and methods

Tissues

In all, 10 tissue specimens with normal gastric antrum and corpus mucosa, chronic corpus gastritis

without atrophy, atrophic corpus gastritis and pancreatic metaplasia of the gastric fundus region were randomly included in the study, respectively. All biopsy samples were submitted to our Institutes of Pathology for routine diagnostic purposes. Gastric mucosal alterations were classified according to the updated Sydney System.[7] To establish the immunohistochemical protocol and to substantiate our findings in the gastric mucosa, 12 normal adult pancreatic tissue samples collected from upper abdominal surgical resection specimens were also analyzed. The age and sex characteristics of the patients in each subgroup are given in Table 1. All specimens were formalin fixed and paraffin embedded according to routine protocols.

Immunohistochemistry

Sections were deparaffinized for 30 min. After rehydration, sections were transferred to 0.1 M citrate buffer, pH 6, and then pretreated in a microwave at 800 W and 500 W for 15 min each. The sections were incubated with a polyclonal rabbit anti-PDX–1 antibody (generously provided by Dr Chris Wright, Vanderbilt University Medical School, Nashville, TN, USA, 1:1000) overnight at room temperature. This PDX-1 antibody was raised against a fusion protein of GST with N-terminal 75 amino acids of mouse PDX-1.[8] It specifically recognizes PDX-1 on Western blots[9,10] and was also used in several immunohistochemical studies in the developing and regenerating pancreas.[11] Binding of the primary antibodies was detected using biotinylated swine anti-rabbit antisera (1:50; Dako, Hamburg, Germany), and a streptavidin–biotinylated alkaline phosphatase complex (Dako). Fast Red (Sigma-Aldrich, Taufkirchen, Germany) was employed as chromogen. Counterstaining was performed using hematoxylin. In addition to the histological diagnosis of pancreatic metaplasia and endocrine cell hyperplasia in routine hematoxylin and eosin (H&E) sections, further immunohistochemistry using rabbit antibodies against amylase (1:2000, Sigma) and mouse antibodies against Chromogranin A (1:1000, Immunotech, Hamburg, Germany) was performed, respectively.

Table 1 Sex and age characteristics of the patients in the subgroups

Subgroup	*Male:female*	*Mean age (range)*
Normal pancreas ($n=12$)	5:7	65 (44–81)
Normal antrum ($n=10$)	4:6	54 (24–81)
Normal corpus ($n=10$)	4:6	53 (24–79)
Non atrophic gastritis ($n=10$)	2:8	64 (42–81)
Atrophic corpus gastritis ($n=10$)	6:4	67 (35–83)
Pancreatic metaplasia ($n=10$)	4:6	52 (29–71)

Double-immunofluorescence Staining

The sections were treated as above and pretreated in a microwave and by coincubating them with 0.1%. Pronase (Sigma) for 5 min. After blocking with 2% goat-serum (Sigma) for 30 min, the primary antibodies were applied overnight in 2% goat serum. The primary antibodies used comprised the rabbit anti-PDX–1 and anti-amylase antibodies (used as mentioned above), a rabbit anti-chromogranin-A antibody (1:1000; Dako, Hamburg, Germany) and a mouse antibody specific for the β-subunit of the gastric H,K-ATPase (1:1000, Alexis, Grünberg, Germany).[12] Then a Cy2-labeled anti-mouse (1:200, Dianova, Hamburg, Germany) and a Cy3-labeled anti-rabbit antibody (1:1500 for PDX-1 and 1:500 for Chromogranin A and Amylase; Dianova) was added for 1 h and kept in darkness. Finally, the nuclei were counterstained with Dapi-Hoechst (1:1000, Molecular Probes, PoortGebouw, Leiden) for 5 min. The following double-immunofluorescence combinations were evaluated: PDX-1/β-H,K-ATPase; chromogranin A/β-H,K-ATPase and amylase/β-H,K-ATPase.

Results

Expression of PDX-1 in Normal Adult Human Pancreas and Gastric Mucosa

In the normal pancreas, PDX-1 was detected in the cytoplasm and nuclei of epithelial cells of small pancreatic ductules in 2/12 and 7/12 cases, respectively (Figure 1a). In single cases, a very weak nuclear staining was visible in acini (Figure 1b), which could correspond to the joining region between ductules and acini and might be associated with increased epithelial regeneration for unknown reasons. Furthermore, PDX-1 was expressed in the cytoplasm of cells in the islets of Langerhans in all specimens. Additionally, a clear nuclear staining was found in most (9/12) cases in the endocrine islets (Figure 1b).

In the normal antrum mucosa, a nuclear and cytoplasmic expression was found in epithelial cells in the neck region of the glands (Figure 1c).

In normal gastric corpus mucosa and in non-atrophic corpus gastritis, PDX-1 was mostly negative or only very weakly detected in the cytoplasm of parietal cells in 3/10 and 2/10 cases, respectively (Figure 1d). The frequency and pattern of PDX-1 expression in normal and diseased gastric mucosa is summarized in Table 2.

Expression of PDX-1 in and Around Pancreatic Metaplasia

In total, 10/10 cases with pancreatic metaplasia were positive for amylase in immunohistochemistry (Figure 2a). In the metaplastic areas, PDX-1 was found in 6/10 cases in the cytoplasm (Figure 2c). In

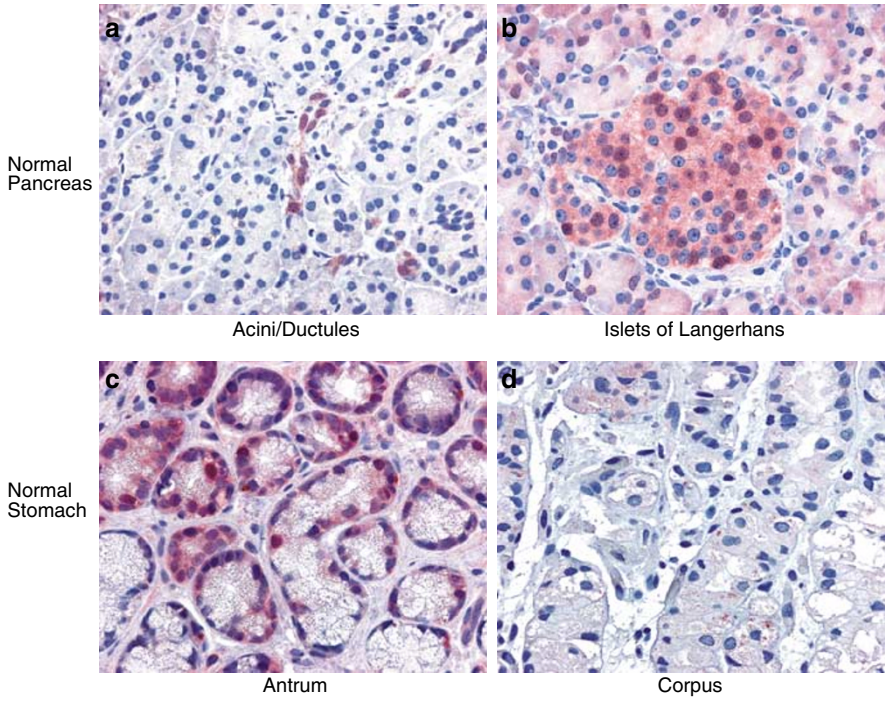

Figure 1 Cytoplasmic and nuclear expression of PDX-1 in ductules (**a**) and islets of Langerhans (**b**) in normal adult pancreas. Nuclear and cytoplasmic expression of PDX-1 in neck cells of normal antrum mucosa (**c**). No PDX-1 expression in normal gastric corpus mucosa (**d**). Immunostaining: original magnification $\times 40$.

4/10 cases, a nuclear staining pattern was found in some of the metaplastic cells and in cells of the adjacent gastric neck region (Figure 2e). Interestingly, and in contrast to normal gastric mucosa and nonatrophic gastritis without metaplasia, the gastric parietal cells in biopsies with pancreatic metaplasia showed moderate to strong immunoreactivity for PDX-1 in all (10/10) biopsies (Figure 2e). Double staining with antibodies against the β-subunit of the gastric H,K-ATPase and with PDX-1 confirmed the parietal cell nature of PDX-1-expressing cells surrounding pancreatic metaplastic nodules (Figure 2g). No coexpression of amylase and the β-H,K-ATPase was found in the metaplastic areas.

Expression of PDX-1 in and Around Endocrine Cell Hyperplasia

In 10/10 cases with gastric mucosa atrophy, a nodular and diffuse endocrine cell hyperplasia was visible and confirmed by immunoreactivity with antibodies against chromogranin A (Figure 2b). In all cases, endocrine cell nodules were immunoreactive with anti-PDX-1 antibodies in the cytoplasm. In 4/10 cases, few endocrine cells in these nodules showed a weak nuclear staining pattern (Figure 2d). Staining intensity was generally weaker than that found in the endocrine islets of the normal pancreas. A nuclear staining pattern for PDX-1 was also found in the neck region of gastric glands adjacent to endocrine nodules in 6/10 cases (Figure 2f). In double-immunofluorescence, diffusely and nodularly arranged hyperplastic endocrine cells showed coexpression of chromogranin A and the β-subunit of the gastric H,K-ATPase (Figure 2h). In contrast, no double-positive cells were observed in normal gastric corpus mucosa (not shown). The staining intensity with the anti-H,K-ATPase antibodies in double-positive cells was generally weaker than that observed in normal parietal cells.

Discussion

In this study, we aimed to analyze the potential role of PDX-1, a key factor in pancreatogenesis, for pancreatic metaplasia and the so-called endocrine cell hyperplasia in the gastric mucosa. To validate the method used, we additionally analyzed normal human pancreatic tissue and found that PDX-1 is mainly expressed in the cytoplasm and nuclei of endocrine and ductular cells in normal adult pancreas. These findings are in good agreement with earlier studies in mice and human pancreata, and therefore substantiate our observations in normal and pathologic gastric mucosa.[11,13] Although PDX-1 acts as a transcription factor, it can also be found in an inactive form in the cytoplasmic compartment. Activation and nuclear translocation in pancreatic cells were shown to be regulated by glucose.[14]

In the normal gastric antrum, PDX-1 expression was found in scattered epithelial cells of the neck

Table 2 PDX-1 expression in normal antrum and corpus mucosa, nonatrophic and atrophic corpus gastritis and in pancreatic metaplasia

Location	*Normal antrum*	*Normal corpus*	*Non-atrophic corpus gastritis*	*Pancreatic metaplasia*			*Atrophic corpus gastritis*			
				Metaplastic areas		*Adjacent parietal cells*	*Endocrine nodules*		*Neck region of adjacent gastric glands*	
				Cytoplasmic	*Nuclear*		*Cytoplasmic*	*Nuclear*	*Cytoplasmic*	*Nuclear*
Frequency	10/10	3/10	2/10	6/10	4/10	10/10	10/10	4/10	10/10	6/10
Comments	Nuclear and cytoplasmic in neck region	Weakly in cyto-plasm of parietal cells			In periphery of metaplasia and in adjacent glands	Strongly in cytoplasm				

region of the glands. In normal body mucosa, PDX-1 was mostly negative. These results are concordant to the findings reported by Larsson *et al*.[3] In contrast to normal gastric mucosa, PDX-1 was found in the cytoplasm and in the nuclei of acinar metaplastic cells and cells adjacent to metaplastic areas in about half of the cases with pancreatic metaplasia. Furthermore, cytoplasmic and nuclear expression of PDX-1 was also present in the hyperplastic endocrine nodules or in the adjacent gastric glands in cases with atrophic body gastritis. We therefore conclude that PDX–1 represents a relevant pathogenic factor for the development of both kinds of gastric glandular alterations, pancreatic metaplasia and the so-called endocrine cell hyperplasia. Therefore, PDX-1 could be regarded as switch factor for endocrine and exocrine differentiation and transdifferentiation processes not only in the pancreatic but also in gastric glands.

In their original description of pancreatic metaplasia, Doglioni *et al*[4] pointed out that the acinar-like metaplastic cells are localized within the same basement membrane and are joined to normal gastric cells by desmosomes, which might indicate that the progenitor cell is part of the normal gastric epithelium. The authors also noticed a striking resemblance of parietal cell and metaplastic cells.[4] In our study, we found that parietal cells surrounding pancreatic metaplasia strongly exhibited a cytoplasmic staining for PDX-1, which is in sharp contrast to parietal cells of the normal body mucosa. This could indicate that an impaired differentiation of the parietal cell lineage might be responsible for the development of pancreatic metaplasia. Nuclear PDX-1 expression was mainly found in the periphery of metaplastic areas or in gastric neck cells adjacent to pancreatic metaplasia. This implies that PDX-1 plays a role in the initiation of metaplasia. Those metaplastic areas in which PDX-1 was not found might represent more differentiated lesions resembling acini in the adult pancreas, which are also PDX-1 negative.[11,13]

With regard to their morphology and function, hyperplastic endocrine nodules in chronic atrophic corpus gastritis resemble in some aspects to endocrine islets of the pancreas. Additionally, with respect to their PDX-1 expression profile, these nodules and the surrounding gastric glands share similarities with pancreatic tissue. This again indicates that PDX-1 might act as an initiating factor for the development of these endocrine cell nodules similar to its role in the formation of Langerhans islets in the pancreas.[2] In all cases tested, we found a coexpression of the gastric H,K-ATPase β-subunit and endocrine markers in these cell nests and glands. Hence, one could speculate that endocrine nodules in atrophic gastritis rather represent areas of 'endocrine pancreatic metaplasia' than truly hyperplastic and non-metaplastic endocrine cells of the stomach. Again cells of the gastric parietal cell lineage appear to be potential precursors. This

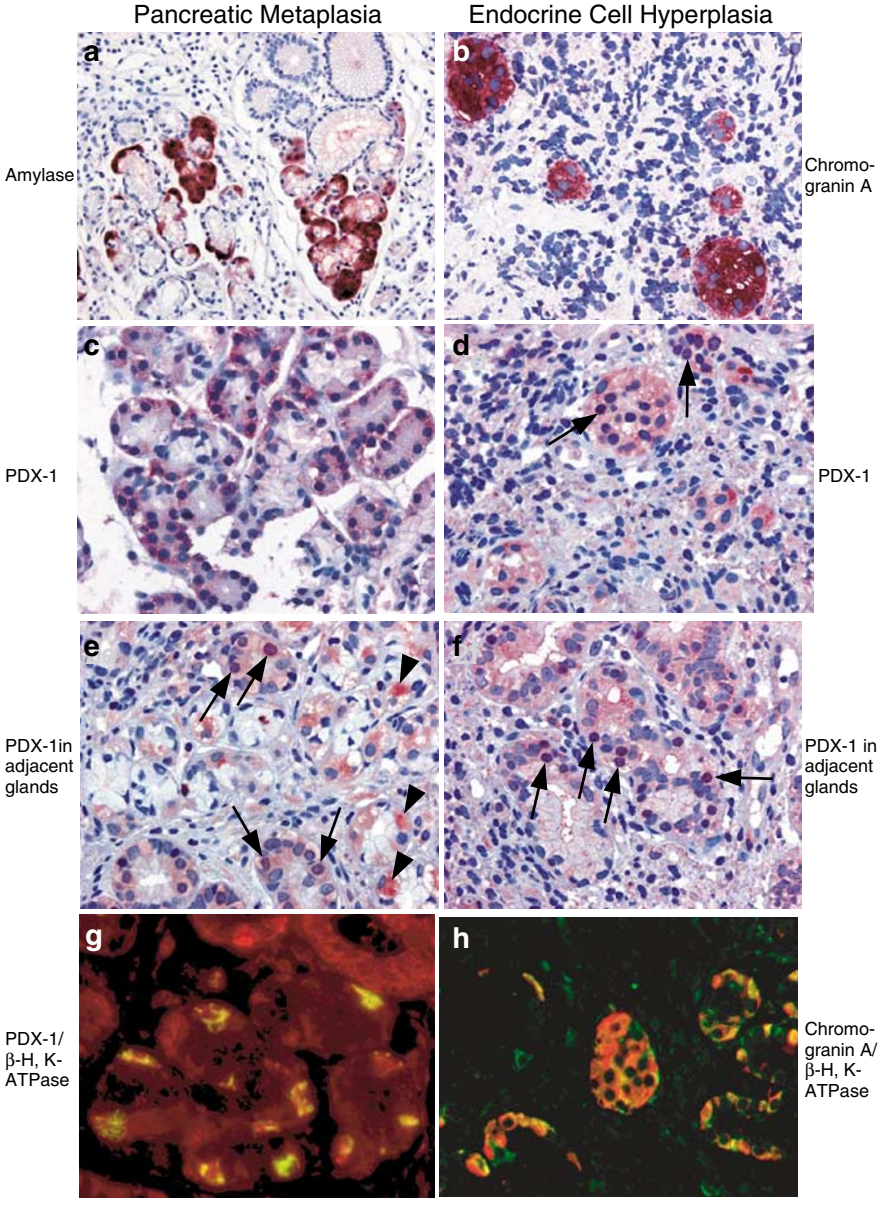

Figure 2 Expression of PDX-1 in pancreatic metaplasia and endocrine cell hyperplasia in atrophic corpus gastritis. Strong amylase reactivity of pancreatic metaplasia (**a**). Cytoplasmic PDX-1 expression in central areas of pancreatic metaplasia (**c**) and nuclear expression (arrows) in the peripheral metaplastic zone and in flanking gastric glands (**e**). Strong cytoplasmic PDX-1 expression in adjacent parietal cells (arrowheads, **e**), which is confirmed by double-immunofluorescence in (**g**) (green: β-H,K-ATPase; red: PDX-1, colocalization: yellow). Strong chromogranin A reactivity in hyperplastic endocrine nodules in atrophic corpus gastritis (**b**). Cytoplasmic and nuclear (arrows) PDX-1 expression in hyperplastic endocrine nodules (**d**) and in adjacent gastric glands (**f**). Coexpression (yellow) of β-H,K-ATPase (green) and chromogranin (red) in hyperplastic endocrine cells (**h**). Immunostaining and double-immunofluorescence. Original magnification: (**a**) $\times$ 20; (**b–h**) $\times$ 40.

would confirm the concept of the gastric parietal cell being the central organizer of the gastric mucosa.[15]

The factors leading to an upregulation, activation and nuclear translocation of PDX-1 in fundic epithelial cells are unknown. The gastric morphogenic factor Sonic hedgehog (Shh) is expressed in the gastric parietal cells and is lost in atrophic gastritis.[15,16] Since Shh acts as negative regulator for PDX-1, it might be possible that a decrease of Shh in gastric atrophy results in the aberrant expression of PDX-1 in the parietal cell lineage.[17,18] This could consequently initiate a new genetic program inducing exocrine and endocrine glandular differentiation in the gastric mucosa similar to embryonic pancreatogenesis. Other factors such as gastrin, inflammatory cytokines, microbial factors or even changes in the gastric acidic milieu could also be responsible for PDX-1 upregulation and expression in the gastric corpus and fundic mucosa. To further investigate these issues, more descriptive and functional analyses are needed.

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References

1 Edlund H. Pancreatic organogenesis—developmental mechanisms and implications for therapy. Nat Rev Genet 2002;3:524–532.
2 Hui H, Perfetti R. Pancreas duodenum homeobox-1 regulates pancreas development during embryogenesis and islet cell function in adulthood. Eur J Endocrinol 2002;146:129–141.
3 Larsson LI, Madsen OD, Serup P, *et al.* Pancreatic—duodenal homeobox 1-role in gastric endocrine patterning. Mech Dev 1996;60:175–184.
4 Doglioni C, Laurino L, Dei Tos AP, *et al.* Pancreatic (acinar) metaplasia of the gastric mucosa. Histology, ultrastructure, immunocytochemistry, and clinicopathologic correlations of 101 cases. Am J Surg Pathol 1993;17:1134–1143.
5 el-Zimaity HM, Verghese VJ, Ramchatesingh J, *et al.* The gastric cardia in gastro-oesophageal disease. J Clin Pathol 2000;53:619–625.
6 Solcia E, Fiocca R, Villani L, *et al.* Hyperplastic, dysplastic, and neoplastic enterochromaffin-like-cell proliferations of the gastric mucosa. Classification and histogenesis. Am J Surg Pathol 1995;19:S1–S7.
7 Dixon MF, Genta RM, Yardley JH, *et al*, Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–1181.
8 Peshavaria M, Gamer L, Henderson E, *et al.* XIHbox 8, an endoderm-specific *Xenopus* homeodomain protein, is closely related to a mammalian insulin gene transcription factor. Mol Endocrinol 1994;8:806–816.
9 Macfarlane WM, Frayling TM, Ellard S, *et al.* Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. J Clin Invest 1999;104:R33–R39.
10 Gannon M, Gamer LW, Wright CV. Regulatory regions driving developmental and tissue-specific expression of the essential pancreatic gene pdx1. Dev Biol 2001;238:185–201.
11 Kritzik MR, Jones E, Chen Z, *et al.* PDX-1 and Msx-2 expression in the regenerating and developing pancreas. J Endocrinol 1999;163:523–530.
12 Chow DC, Forte JG. Characterization of the beta-subunit of the H(+)-K(+)-ATPase using an inhibitory monoclonal antibody. Am J Physiol 1993;265:C1562–C1570.
13 Heimberg H, Bouwens L, Heremans Y, *et al.* Adult human pancreatic duct and islet cells exhibit similarities in expression and differences in phosphorylation and complex formation of the homeodomain protein Ipf-1. Diabetes 2000;49:571–579.
14 Macfarlane WM, McKinnon CM, Felton-Edkins ZA, *et al.* Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. J Biol Chem 1999;274:1011–1016.
15 van den Brink GR, Hardwick JC, Tytgat GN, *et al.* Sonic hedgehog regulates gastric gland morphogenesis in man and mouse. Gastroenterology 2001;121:317–328.
16 van den Brink GR, Hardwick JC, Nielsen C, *et al.* Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. Gut 2002;51:628–633.
17 Hebrok M, Kim SK, St Jacques B, *et al.* Regulation of pancreas development by hedgehog signaling. Development 2000;127:4905–4913.
18 Kim SK, Hebrok M. Intercellular signals regulating pancreas development and function. Genes Dev 2001;15:111–127.