PATHOLOGICA 2014;106:315-321

Review

PDX-1 (Pancreatic/Duodenal Homeobox-1 Protein 1)

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Key words

PDX-1 • Immunoistochemistry • Pancreatic development • Normal human tissues

Summary

The homeodomain-containing transcription factor pancreatic duodenal homeobox 1 (PDX-1) plays a key role in pancreatic development and β-cell function. It is a major regulator of transcription in pancreatic cells, and transactivates the insulin gene by binding to a specific DNA motif in its promoter region. Glucose also regulates insulin gene transcription through PDX-1.
It has been shown that PDX-1 is required for maintaining pancreatic islet functions by activating gene expression and has a dual role in pancreatic development. It initially contributes to pancreatic formation during embryogenesis and subsequently regulates the pancreatic islet cell physiology in mature islet cells.
Because of this key role in the embryologic development of the pancreas, PDX-1 expression has been investigated in pancreatic cancer cell lines and human tumors.
Moreover, a few reports have described expression of PDX-1 in other human neoplasms and have investigated its potential role in differential diagnosis, but data on normal human tissues are lacking. Understanding the molecular mechanisms of pancreas formation, and especially the function of PDX-1, may contribute to the improved treatment and prevention of debilitating diseases such as diabetes, insulinomas and pancreatic carcinomas. Nevertheless, further studies are needed concerning its possible application in routine practice.

Introduction

Pancreatic compartments have been demonstrated to derive from progenitor cells that express the pancreatic and duodenal homeobox gene (PDX-1) during pancreas development [1]. The human pancreatic and duodenal homeobox-1 gene (PDX-1) is located on chromosome 13q12.1 near the CDX2 gene [2]. In mouse and rat, the PDX-1 genes are localized on chromosomes 5 [3], and 12 [4], respectively.
PDX-1 (also known as IDX-1/STF-1/IPF1) [5 6] is a marker of all pancreatic and midgut progenitors, is expressed in precursors of the endocrine and exocrine (acinar and duct) compartments of the pancreas and is essential for development of the pancreas [7]. PDX-1 is also expressed in the adjacent presumptive stomach and duodenum [5 7].
This gene belongs to a Para-Hox gene cluster out of the major Hox cluster of homeodomain proteins 1 and its coding region comprises two exons [8]: the first encodes for the NH2-terminal region of PDX-1 and the second for the homeodomain and COOH-terminal domain. The activation domain is located in the NH2-terminal region, while the homeodomain is responsible for DNA binding [9 10].
PDX-1 is a pancreas-specific homeoprotein, β - and δ-cell -specific and responsible for transcription and expression of insulin and somatostatin. PDX-1 activity is central to the regulation of a number of glucoregulatory genes within the β-cells, including insulin [11], islet amyloid polypeptide (IAPP) [12], glucose transporter type 2 (GLUT2) [13] and glucokinase [14]. It regulates the balance between the exocrine (acinar and ducts) and endocrine progenitors that differentiate within the pancreas [15], depending on glucose levels through phosphorylation [16] and nuclear translocation [17].
Anatomical observations of amniotic embryos demonstrated that pancreas progenitors develop from a segment of the dorsal endoderm and separately from paired lateral domains of endoderm.
When the gut-tube closes, the lateral pancreatic endoderm domains fuse at the ventral region of the gut to form the ventral pancreatic bud, while the dorsal pancreatic endoderm goes on to form the dorsal pancreatic bud. In the end the two buds fuse with the ventral descendants

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populating part of the "head" of the pancreas and the dorsal descendants forming the rest of the gland [18]. The ventral bud develops adjacent to the hepatic diverticulum, while the dorsal bud arises on the opposite side of the gut tube. When the stomach and duodenum rotate, the ventral bud and hepatopancreatic orifice rotate and fuse with the dorsal bud. The ventral bud forms the uncinate process, while the dorsal bud forms the other part of the pancreas. The ventral duct fuses with the distal part of the dorsal duct to become the main duct of Wirsung and the proximal part of the dorsal duct becomes the duct of Santorini [18].

Mice homozygous for a targeted mutation in PDX-1 selectively lack a pancreas, but the duodenum has a normal C-shaped form [7]. In homozygous mutants there is no pancreatic duct, but the common bile duct is present [7]. PDX-1 knockout mice exhibit pancreatic agenesis and abnormal formation of the pylorus and duodenum [7 19 20]. Defects in this gene are a cause of pancreatic agenesis, which can lead to early-onset insulin-dependent diabetes mellitus, as well as maturity onset diabetes of the young type 4 (MODY4) [21].

PDX-1 is first detected at embryonic day 8.5 (E8.5) in the dorsal endoderm of the murine gut when it is still an open tube and expressed in the dorsal and ventral pancreatic buds and in the intervening endoderm of the presumptive duodenum at E9.5 [21]. Its high expression is maintained in most epithelial cells of the pancreatic bud until E10.5, after which it decreases and is present later in the differentiated β-cell [22].

When the pancreatic epithelium proliferates and invades the mesenchyme around, the mesenchyme itself sends signals to the pancreatic epithelium to promote cellular differentiation and morphogenesis. In fact, without mesenchymal signals epithelial cells fail to grow and acini do not form [23]. In PDX-1-null mice the pancreatic mesenchyme forms a hollow bud-like structure without epithelium [24].

Beyond β- and δ-cells, a lower expression of PDX-1 is present in the pancreatic acinar cells, epitelium of the duodenum, Brunner's glands of the duodenum and pyloric glands of the stomach [21 25].

Miyatsuka T et al. demonstrated that persistent expression of PDX-1 induces acinar-to ductal metaplasia in a cell-autonomous manner [26]. This occurs because up-regulation of PDX-1 causes activation of signal transducer and activator of transcription (STAT-3), which has been described in mouse models of pancreatic metaplasia [27].

Fukuda et al. [13] demonstrated that PDX-1 inactivation leads to loss of the major duodenal papilla and formation of brown pigment stones in the common bile duct. They also showed that PDX-1 null mice do not develop peribiliary glands or mucin-producing cells in the common bile duct. On the other hand, its re-upregulation has been reported in human patients and several mouse models of pancreatic cancer and pancreatitis [28-30].

Extrahepatic bile ducts derive from PDX-1 positive cells in the foregut endoderm, sharing the common origin with the ventral pancreas but not with the liver [31], while the intrahepatic biliary cells derive from hepatoblasts. The liver itself derives from the ventral foregut and it needs signals from the cardiac mesoderm (such as fibroblast growth factor, FGF) [32] to develop [33].

The endoderm contains the precursors that give rise to the epithelium of both the gut and associated organs, such as the liver, pancreas and respiratory tract. In fact, the respiratory system arises from the ventral foregut endoderm [34].

PDX-1 and diabetes

Thomas IH et al. [35] described the combination of severe exocrine pancreatic insufficiency and permanent neonatal diabetes which suggested the possibility of pancreatic agenesis and, by association, the presence of a PDX-1 mutation.

Regarding acquired diabetes, Macfarlane et al. [36] identified 3 mutations in the β-cell transcription factor PDX-1 associated with type 2 diabetes. All 3 mutations (C18R, D76N and R197H) resulted in reduced binding of the protein to the insulin gene promoter and decreased insulin gene transcription.

Considering possible new strategies in treatment of diabetes mellitus, Yuan et al. [37] recently generated mesenchymal stem cells that are able to secrete insulin with stable transfection of the PDX-1 gene. The authors demonstrated that overexpression of PDX-1 in mesenchymal stem cells alone is sufficient in induction of insulin gene expression and insulin secretion. Another group [38] developed a strategy to generate human insulin producing cells using a 3D culture system with peripheral blood cells.

There is promising progress in redirecting various cell types to behave like β-cells and to produce insulin. However, in-depth knowledge of post-translational modifications of the PDX-1 protein and its interaction with other regulatory proteins will be fundamental to develop new treatments for diabetes mellitus [39].

PDX-1 as a target for anti-cancer therapy

Recently, Wu et al. [40] have described the possibility to utilise Pdx-1 as a target gene for pancreatic cancer. In fact, Pdx-1 has been described as a potential molecular target for pancreatic cancer [41 42] and the authors [40] have recently described the possibility to use RNA interference (RNAi) as a powerful new tool for targeted gene therapy.

PDX-1 expression in human normal and neoplastic tissues

Only a few reports investigated the immunoexpression of PDX-1 in human tissues, most of them focusing on gastrointestinal tissue (Tab. I).

Buettner et al. [43] investigated 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 [43]. In fact, although PDX-1 acts as a transcription factor, it can also be found in an inactive form in the cytoplasmic compartment since activation and nuclear translocation in pancreatic cells is regulated by glucose [36].

Tab. I. Literature review table on PDX-1.

Authors	Antibody	Subject	Results (PDX-1 staining)
Buettner[43] et al., 2004	Polyclonal rabbit anti-PDX–1 antibody (see reference for details, 1:1000)	Atrophic corpus in gastritis	4/10 areas of pancreatic metaplasia and parietal cells adjacent to these areas endocrine nodules in 10/10 cases
Sakai[25] et al., 2004	Produced by the authors	Normal and neoplastic stomach	pseudopyloric glands and intestinal metaplasia. differentiated type carcinomas (39/43, 90.7%) T1 carcinomas (42/43, 97.7%) undifferentiated type (33/52, 63.5%) T2–4 (30/52, 57.7%) carcinomas.
Leys[44] et al., 2006	The guinea pig anti–PDX-1 polyclonal antibody [Gastroenterology 2005;128:1292 - 305] .	Gastric adenocarcinoma	antral glands 47/104 gastric fundic cancers 23/46 gastric antral cancers
Liu[41] et al., 2007	Goat anti-PDX-1 monoclonal antibody (Santa Cruz, USA).	Pancreatic cancer	41.1% of pancreatic cancer samples (especially at the leading edge of tumor), correlating with grading
Ballian[46] et al., 2008	Rabbit polyclonal antibody against the N-terminal of PDX-1 peptide [4]	Several human tissues	In this report, levels of PDX-1 expression were quantified in a primary colorectal tumour, a metastasis and in benign tissue from a single patient
Srivastava[47] et al., 2009	PDX-1 Goat Polyclonal (1:100 Microwave; Santa Cruz Biotechnology, CA)	Well differentiated neuroendocrine tumours	60% in stomach 80% in duodenum 0% in ileum and lung 55% in appendix 17% in rectum 28% in pancreas
Park[45] et al., 2011	PDX-1 mouse monoclonal antibody that recognizes the C-terminus of PDX-1 (amino acids 91 to 283) MAB2419, clone 267712; R&D Systems, Minneapolis, MN)	Pancreatic neoplasms	40.6% of PanIn in more than 50% of cells 35.2% of IPMN in more than 50% of cells 2/3 mucinous cystic neoplasms 9/67 pancreatic adenocarcinomas 47.7% of well differentiatied endocrine tumours
Chan[48] et al., 2012	PDX-1 Santa Cruz (sc-14662, Polyclonal goat 1:50)	Neuroendocrine tumours	pancreas 72% bronchopulmonary 10% appendix 17% cecum, colon, ileum and rectum 0%

These Authors [43] found that in normal antrum mucosa, nuclear and cytoplasmic expression of PDX-1 is found in epithelial cells in the neck region of the glands, while in normal body mucosa PDX-1 was mostly negative. Moreover, the gastric parietal cells in biopsies with pancreatic metaplasia had moderate to strong immunoreactivity for PDX-1, which was also found in the cytoplasm and in the nuclei of metaplastic acinar cells as well as cells adjacent to metaplastic areas in about half of cases with pancreatic metaplasia. PDX-1 was also present in the cytoplasm and nucleus of hyperplastic endocrine nodules and in the adjacent gastric glands in cases with atrophic body gastritis. Thus, PDX-1 may represent an important pathogenic factor for the development of pancreatic metaplasia and endocrine cell hyperplasia.

Leys CM et al. [44] showed that fundic mucosa was devoid of cells with true nuclear PDX-1 immunoreactivity, even if normal antral mucosa had nuclear staining for PDX-1, especially in cells at the base of the glands. In case of antralisation of the gastric fundus, PDX-1 expression was not present, but intestinal metaplasia stained strongly for nuclear PDX-1. Nuclear PDX-1 expression was observed in 50% of antral-derived cancers and 40% of fundic cancers. PDX-1 expression did not correlate with clinical outcome [44].

Sakai et al. [25] studied PDX-1 expression in 30 of 39 corpus tumors and intestinal metaplasia. The authors [25] hypothesised that intestinal metaplasia may develop due to formation of pseudopyloric glands in the corpus because PDX-1 together with MUC6 (marker of pseudopyloric metaplasia) is significantly higher in well differentiated carcinomas than undifferentiated type. Thus, they suggested that intestinal metaplasia and differentiated type carcinomas arise on the basis of pseudopyloric/pyloric glands [25].

Park et al. [45] studied PDX-1 expression in pancreatic

cancer and precursor lesions. PDX-1 nuclear labeling was present in non-neoplastic islet cells, centroacinar cells and intralobular and interlobular ducts. PDX-1 was strongly expressed in precursor lesions of pancreatic adenocarcinoma such as intraductal papillary mucinous neoplasm (35.2%), pancreatic intraductal tumours (40.6%) and mucinous neoplasms (2 of 3 cases), but the degree of dysplasia was not correlated with the intensity of staining of PDX-1.

Pancreatic adenocarcinoma had variable positivity (13.4% with strong positivity) and well differentiated neuroendocrine tumors were positive in 38.6% of cases [45].

Ballian et al. [46] reported that PDX-1 expression in 10 colon cancer specimens was significantly elevated in both the nucleus and cytoplasm of malignant cells, with lower levels found in benign tissues. In the same report, the authors [46] found high PDX-1 protein levels in metastases. Two different reports about the diagnostic utility of PDX-1 in neuroendocrine tumors (NET) found somewhat discrepant results in gastrointestinal tract endocrine tumors.

Srivastava et al. [47] observed PDX-1 expression in both gastrointestinal and pancreatic NET, a subset of rectal NET and more than half from the appendix. PDX-1 was absent in ileal and pulmonary NET. They concluded that PDX-1 can be used together with other 3 markers (NE-SP-55, Cdx-2 and TTF-1) to distinguish the origin of well-differentiated NET in the gastro-enteric-pancreatic axis.

In another report, Chan et al. [48] found that PDX-1 was positive in 72% of pancreatic NET, 10% of bronchopulmonary and 4% of GI tract NET. They concluded that PDX-1 in combination with Cdx-2, TTF-1 and CK7, which may help in defining the primary site of origin of NET. PDX-1 was specific and moderately sensitive for pancreatic NET, while CDX-2 was very specific and sensitive for gastrointestinal NET; for a similar pattern was seen for TTF-1 in bronchopulmonary NET.

Park [45] et al studied PDX-1 in gallbladder, liver, prostate, kidney, ovary, spleen, thyroid, lung, breast, cerebellum, tonsils, colon and placenta and found no staining. The authors [45] described weak staining in the basal layer of the skin.

Materials and methods

We collected routinely available normal human tissues from stomach (fundus, corpus, antrum), duodenum, colon, appendix, liver, gallbladder, pancreas, tonsil, spleen, thymus, lung, thyroid, breast, skin, prostate, seminal vescicles, bladder, lymph node, kidney, adrenals, pituitary gland, ovary, uterus, salivary glands and cardiac muscle.

We performed immunohistochemistry, immunofluorescence and Western blot analysis in human normal tissue sections using previously described reagents and protocols [49].

Immunohistochemical analysis

We used a rabbit monoclonal antibody specific to a synthetic peptide of 46 kDa, corresponding to residues on the C-terminus in human PDX-1 antibody (Epitomics, clone EPR3358(2)), which was diluted 1:3000 and antigen retrieval was performed by incubation in buffer ER2 pH 8–9 for 15 min at 95°C. Detection was performed by a polymer-based system (Bond Polymer Refine Detection, Leica Biosystems, Nussloch, Germany) with an automated stainer (Leica Bond-Max).

Moreover, we used chromogranin A antibody (mouse monoclonal, Dako: dak 3, 1:2500) and cytokeratin 7 (OV-TL12/30, BioGenex, 1:400) to perform double immunohistochemical staining and double immunofluorescence analyses.

Immunofluorescence analysis

Sections (3 μm) were collected on polarised slides and let dry for 1 hour at 60°C, and then deparaffinized in xylene for 20 min. Next, sections were hydrated with 100%, 85% and 75% ethanol and rinsed in distilled water. Furthermore, sections were treated for antigen retrieval with citrate buffer at pH 6, previously heated at100°C for 30 min. Afterwards, sections were washed in running water and distilled water, then incubated with protein block solution for 10 min. The protein blocking solution in excess was eliminated and incubated with primary antibody for 1 hour at room temperature in a wet room; they were then washed 3 times with PBS and incubated with secondary antibody conjugated with fluorochrome for 30 minutes in a wet dark room at room temperature.

Later, sections were washed with PBS and incubated with alcoholic solution with 0.5% Sudan black for 10 min and then washed again with PBS. Excess PBS was eliminated and 20 μl of DAPI were added directly before application of a cover slip. The procedure was repeated for the second antibody. Sections were then examined with fluorescence microscope.

First primary antibody staining sites were visible in green, second primary antibody staining sites in red and double staining sites in yellow. Counterstained nuclei were visible in blue light. We applied PDX-1 (1:500) and chromogranin (1:2000) antibodies.

Western blot analysis

For each sample, 20 serial 10 μm sections of fresh frozen tissue were collected in an Eppendorf tube; 150 μl of cell lysis buffer (Cell Signaling Technology) was added prior to heating at 100°C for 5 min. Samples were cooled for 5 min on ice, centrifuged at 14,000 × g for 15 min and supernatants were transferred to a fresh tube and stored at –20°C.

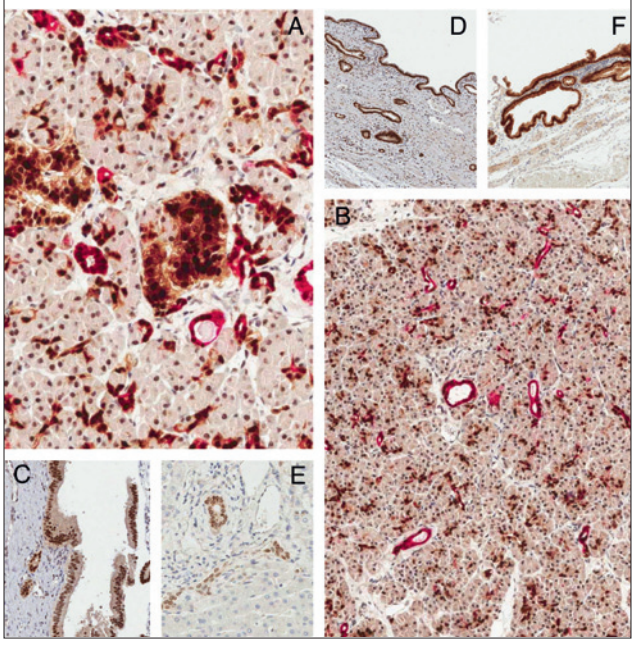
Protein quantification was performed by using the Bio-Rad protein assay kit according to manufacturer's instructions. 25 μg of extracted lysates was resolved on a 10% polyacrylamide SDS-PAGE gel in a BioRad Mini Protean tetra cell system at 150 V for 1 h.

Electrophoresed proteins were transferred onto a nitrocellulose membrane at 250 mA for 90 min. Membranes were blocked in TBST plus 5% non-fat dry milk for 1 h at RT with constant shaking. They were incubated overnight at 4°C with the indicated antibodies, washed three times with TBST and incubated with the specific secondary anti-mouse or anti-rabbit peroxidase-conjugated anti IgG antibody. After three washes with TBST, immunoblots were visualized with ECLplus (Amersham/GE Healthcare Europe GmgH, Munich, Germany).
Expression levels of PDX-1 were quantified by ImageJ densitometric analysis. An anti-β-actin antibody (ab6276, Abcam, Cambridge, UK) was used as a control for protein loading.

Results

We found PDX-1 to have well defined nuclear staining and to be heterogeneously expressed only in the digestive tract with some differences in the different organs. In particular, we confirmed previous results since in the pancreas PDX-1 stained normal endocrine islets (Fig. 1A), pancreatic ducts and ductules (Fig. 1 A, B), but not acini. Moreover, both the Wirsung duct and intrapancreatic bile duct were positive (Fig. 1 C, D) showing a strong staining nuclear pattern.
In the liver, PDX-1 stained the bile duct epithelium of the major and minor branches of the biliary tree and peribiliary glands (Fig. 1E), but was not expressed in normal hepatocytes. Moreover, PDX-1 was widely and strongly expressed in the gallbladder epithelium (Fig. 1F).
In the gut tube, PDX-1 was strongly expressed in the mucosa of antrum (Fig. 2A) and duodenum (Fig. 2B),

Fig. 1. Immunohistochemical staining of PDX-1 in the pancreas and biliary tree.



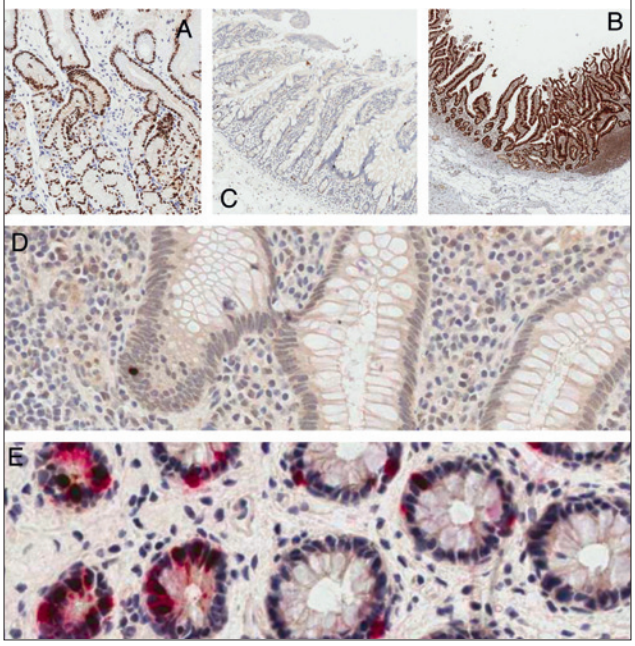


Fig. 2. Immunohistochemical staining of PDX-1 in the gut.

but not in the rest of the stomach or in the oesophagus. The ileal (Fig. 2C), appendiceal (Fig. 2D) and colonic mucosa (Fig. 2E) expressed PDX-1 in only a few scattered cells. These PDX-1 positive cells tended to be located at the base of the crypts and were characterised by a fusiform small nucleus with scant cytoplasm. Some of these scattered PDX-1 positive cells co-expressed chromogranin (Figs. 2E and 3).
Moreover, we found weak staining for PDX-1 in the adrenal gland and granulosa cells of the ovary, although Western blot analyses showed these were false positive cases, confirming the results in colon (Fig. 4). All other tissues were negative for PDX-1.

Conclusions

PDX-1 is expressed in the human digestive tract, and in particular in the duodenal and duodenal-pancreatic district. In the liver, PDX-1 stains only the biliary tree, but hepatocytes are negative.
These results can be explained by the embryology of pancreas and liver since they both develop from the ventral pancreatic bud [18]. Moreover, along the rest of the digestive tube, it stains scattered small cells in the small intestine (other than duodenum) and large bowel. The differences between duodenual staining and the rest of the intestine can also be explained by embryology.
The duodenum arises from two adjacent regions of the gut tube: the foregut and midgut. The junction between these two regions lies at the mid-point of the duodenum, at the level of the entry of the bile duct. These cells are worthy of further investigation because they some co-stain chromogranin and some do not. This peculiar char-

Fig. 3. Immunofluorescence in colonic mucosa with chromogranin (red) and PDX-1 (brown).

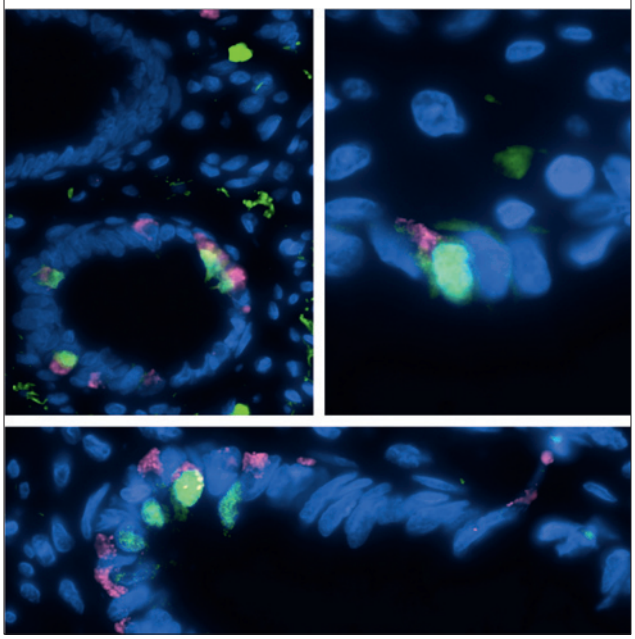
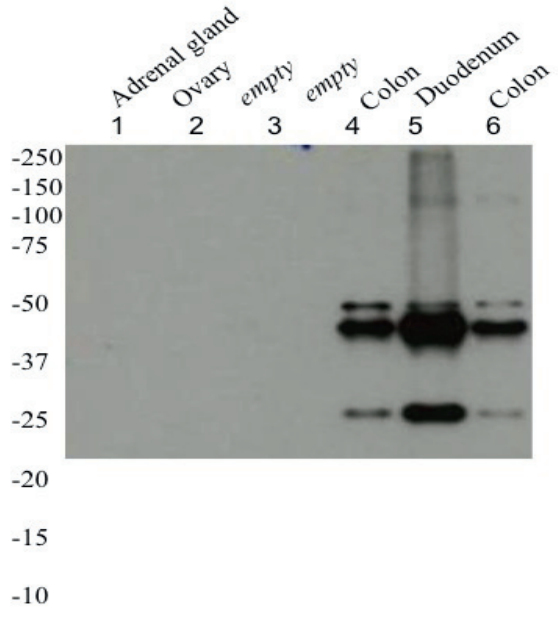


Fig. 4. Western blot analysis of PDX-1.



acteristic, together with their morphology and anatomical location, may suggest that they could represent the "stem cell" niche of the intestine.

The endoderm also forms the lining of three accessory organs that develop immediately caudal to the stomach. The hepatic diverticulum is the tube of endoderm that extends out from the foregut into the surrounding mesenchyme. The mesenchyme induces this endoderm to proliferate, to branch and to form the glandular epithelium of the liver. A portion of the hepatic diverticulum (the region closest to the digestive tube) continues to function as the drainage duct of the liver, and a branch from this duct produces the gallbladder [18].

PDX-1 expression marks a pluripotent population of cells that give rise to all cell types of the neonatal pancreas (endocrine, exocrine and duct) and epithelium of the duodenum and posterior stomach [1]. These data can explain the different distribution of PDX-1 expression among the gut tube.

Since PDX-1 is restricted to a few precise districts, it can be useful in suggesting the origin of endocrine neoplasms when unknown, but more data are needed to demonstrate its sensibility and specificity in routine practice. Until now, only 3 reports have investigated PDX-1 expression in well differentiated neuroendocrine tumours [45 47 50], but they applied 3 different PDX-1 antibodies.

Moreover, our antibody was different from those reported in the literature and demonstrated to have a true positivity when it detected strong and precise nuclear staining, while other reports [43 46] also considered cytoplasmic staining with different clones as true positivity.

The common result presented herein is that the PDX-1 gene expressed specifically in the duodenum and biliopancreatic tree and is a valid marker for this district. More reports are needed comparing different PDX-1 antibodies to better define which is best in term of sensitivity and specificity.

References

1 Gu G, Dubauskaite J, Melton DA. *Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors.* Development 2002;129:2447-57.

2 Brooke NM, Garcia-Fernandez J, Holland PW. *The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster.* Nature 1998;392:920-2.

3 Sharma S, Leonard J, Lee S, et al. *Pancreatic islet expression of the homeobox factor STF-1 relies on an E-box motif that binds USF.* J Biol Chem 1996;271:2294-9.

4 Yokoi N, Serikawa T, Walther R. *PDX-1, a homeodomain transcription factor required for pancreas development, maps to rat chromosome 12.* Exp Anim 1997;46:323-4.

5 Ohlsson H, Karlsson K, Edlund T. *IPF1, a homeodomain-containing transactivator of the insulin gene.* EMBO J 1993;12:4251-9.

6 Leonard J, Peers B, Johnson T, et al. *Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells.* Mol Endocrinol 1993;7:1275-83.

7 Jonsson J, Carlsson L, Edlund T, et al. *Insulin-promoter-factor 1 is required for pancreas development in mice.* Nature 1994;371:606-9.

8 Stoffel M, Stein R, Wright CV, et al. *Localization of human homeodomain transcription factor insulin promoter factor 1 (IPF1) to chromosome band 13q12.1.* Genomics 1995;28:125-6.

9 Marshak S, Benshushan E, Shoshkes M, et al. *Functional conservation of regulatory elements in the PDX-1 gene: PDX-1 and hepatocyte nuclear factor 3beta transcription factors mediate beta-cell-specific expression.* Mol Cell Biol 2000;20:7583-90.

10 Asahara H, Dutta S, Kao HY, et al. *Pbx-Hox heterodimers recruit*

coactivator-corepressor complexes in an isoform-specific manner. Mol Cell Biol 1999;19:8219-25.

[11] Docherty K, Clark AR. *Nutrient regulation of insulin gene expression*. FASEB J 1994;8:20-7.

[12] Serup P, Jensen J, Andersen FG, et al. *Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1.* Proc Natl Acad Sci U S A 1996;93:9015-20.

[13] Waeber G, Thompson N, Nicod P, et al. *Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor.* Mol Endocrinol 1996;10:1327-34.

[14] Watada H, Kajimoto Y, Umayahara Y, et al. *The human glucokinase gene beta-cell-type promoter: an essential role of insulin promoter factor 1/PDX-1 in its activation in HIT-T15 cells.* Diabetes 1996;45:1478-88.

[15] Fujitani Y, Fujitani S, Boyer DF, et al. *Targeted deletion of a cis-regulatory region reveals differential gene dosage requirements for PDX-1 in foregut organ differentiation and pancreas formation.* Genes Dev 2006;20:253-66.

[16] Petersen HV, Peshavaria M, Pedersen AA, et al. *Glucose stimulates the activation domain potential of the PDX-1 homeodomain transcription factor.* FEBS Lett 1998;431:362-6.

[17] Rafiq I, Kennedy HJ, Rutter GA. *Glucose-dependent translocation of insulin promoter factor-1 (IPF-1) between the nuclear periphery and the nucleoplasm of single MIN6 beta-cells.* J Biol Chem 1998;273:23241-7.

[18] Slack JM. *Developmental biology of the pancreas.* Development 1995;121:1569-80.

[19] Offield MF, Jetton TL, Labosky PA, et al. *PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum.* Development 1996;122:983-95.

[20] Larsson LI, Madsen OD, Serup P, et al. *Pancreatic-duodenal homeobox 1 -role in gastric endocrine patterning.* Mech Dev 1996;60:175-84.

[21] Guz Y, Montminy MR, Stein R, et al. *Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny.* Development 1995;121:11-8.

[22] Melloul D, Marshak S, Cerasi E. *Regulation of PDX-1 gene expression*. Diabetes 2002;51 Suppl 3:S320-5.

[23] Golosow N, Grobstein C. *Epitheliomesenchymal interaction in pancreatic morphogenesis*. Dev Biol 1962;4:242-55.

[24] Ahlgren U, Jonsson J, Edlund H. *The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX-1-deficient mice.* Development 1996;122:1409-16.

[25] Sakai H, Eishi Y, Li XL, et al. *PDX-1 homeobox protein expression in pseudopyloric glands and gastric carcinomas.* Gut 2004;53:323-30.

[26] Miyatsuka T, Kaneto H, Shiraiwa T, et al. *Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation.* Genes Dev 2006;20:1435-40.

[27] Fukuda A, Kawaguchi Y, Furuyama K, et al. *Loss of the major duodenal papilla results in brown pigment biliary stone formation in PDX-1 null mice.* Gastroenterology 2006;130:855-67.

[28] Song SY, Gannon M, Washington MK, et al. *Expansion of PDX-1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha.* Gastroenterology 1999;117:1416-26.

[29] Koizumi M, Doi R, Toyoda E, et al. *Increased PDX-1 expression is associated with outcome in patients with pancreatic cancer.* Surgery 2003;134:260-6.

[30] Jensen JN, Cameron E, Garay MV, et al. *Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration.* Gastroenterology 2005;128:728-41.

[31] Spence JR, Lange AW, Lin SC, et al. *Sox17 regulates organ lineage segregation of ventral foregut progenitor cells.* Dev Cell 2009;17:62-74.

[32] Jung J, Zheng M, Goldfarb M, et al. *Initiation of mammalian liver development from endoderm by fibroblast growth factors.* Science 1999;284:1998-2003.

[33] Gualdi R, Bossard P, Zheng M, et al. *Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control.* Genes Dev 1996;10:1670-82.

[34] Angelo JR, Guerrero-Zayas MI, Tremblay KD. *A fate map of the murine pancreas buds reveals a multipotent ventral foregut organ progenitor.* PLoS One 2007;7:e40707.

[35] Thomas IH, Saini NK, Adhikari A, et al. *Neonatal diabetes mellitus with pancreatic agenesis in an infant with homozygous IPF-1 Pro63fsX60 mutation.* Pediatr Diabetes 2009;10:492-6.

[36] 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:R33-9.

[37] Yuan H, Li J, Xin N, et al. *Expression of PDX-1 mediates differentiation from mesenchymal stem cells into insulin-producing cells.* Mol Biol Rep 2010;37:4023-31.

[38] Hur J, Yang JM, Choi JI, et al. *New method to differentiate human peripheral blood monocytes into insulin producing cells: human hematosphere culture.* Biochem Biophys Res Commun 2012;418:765-9.

[39] Al-Quobaili F, Montenarh M. *Pancreatic duodenal homeobox factor-1 and diabetes mellitus type 2 (review).* Int J Mol Med 2008;21:399-404.

[40] Wu J, Liu S, Yu J, et al. *Vertically integrated translational studies of PDX-1 as a therapeutic target for pancreatic cancer via a novel bifunctional RNAi platform*. Cancer Gene Ther 2014;21:48-53.

[41] Liu SH, Patel S, Gingras MC, et al. *PDX-1: demonstration of oncogenic properties in pancreatic cancer.* Cancer 2011;117:723-33.

[42] Liu SH, Rao DD, Nemunaitis J, et al. *PDX-1 is a therapeutic target for pancreatic cancer, insulinoma and islet neoplasia using a novel RNA interference platform.* PLoS One 2012;7:e40452.

[43] Buettner M, Dimmler A, Magener A, et al. *Gastric PDX-1 expression in pancreatic metaplasia and endocrine cell hyperplasia in atrophic corpus gastritis*. Mod Pathol 2004;17:56-61.

[44] Leys CM, Nomura S, Rudzinski E, et al. *Expression of PDX-1 in human gastric metaplasia and gastric adenocarcinoma.* Hum Pathol 2006;37:1162-8.

[45] Park JY, Hong SM, Klimstra DS, et al. *PDX-1 expression in pancreatic precursor lesions and neoplasms.* Appl Immunohistochem Mol Morphol 2011;19:444-9.

[46] Ballian N, Liu SH, Brunicardi FC. *Transcription factor PDX-1 in human colorectal adenocarcinoma: a potential tumor marker?* World J Gastroenterol 2008;14:5823-6.

[47] Srivastava A, Hornick JL. *Immunohistochemical staining for CDX-2, PDX-1, NESP-55, and TTF-1 can help distinguish gastrointestinal carcinoid tumors from pancreatic endocrine and pulmonary carcinoid tumors.* Am J Surg Pathol 2009;33:626-32.

[48] Chan ES, Alexander J, Swanson PE, et al. *PDX-1, CDX-2, TTF-1, and CK7: a reliable immunohistochemical panel for pancreatic neuroendocrine neoplasms.* Am J Surg Pathol 2012;36:737-43.

[49] Munari E, Rinaldi M, Ambrosetti A, et al. *Absence of TCL1A expression is a useful diagnostic feature in splenic marginal zone lymphoma.* Virchows Arch 2012;461:677-85.

[50] Chan ES, Alexander J, Swanson PE, et al. *PDX-1, CDX-2, TTF-1, and CK7: a reliable immunohistochemical panel for pancreatic neuroendocrine neoplasms.* Am J Surg Pathol 2012;36:737-43.