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Expression and clinicopathological characteristics of PDX1, PTF1A, and SALL4 in large and small ducts of ectopic pancreas located in gastro-duodenum and jejunum

Mengxi Chen [a], Takehiro Tanaka [a,*], Takuro Igawa [a], Yanyan Han [a], Fangli Peng [a], Zaishun Jin [b], Tadashi Yoshino [a]

[a] Patholothy and Onology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, 700-8558, Japan
[b] Department of Pathology, Mudanjiang Medical University, Mudanjiang, 157001, China

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ABSTRACT

An ectopic pancreas is defined as pancreatic tissue outside its normal location, anatomically separated from the pancreas.

The transcription factor pancreas/duodenum homeobox protein 1 (PDX1) is involved in maintaining the pancreas and functions in early pancreatic development, beta cell differentiation, and endocrine non beta cells. Pancreatic transcription factor 1 subunit alpha (PTF1A) affects exocrine cell formation and regulation of acinar cell identity, and is expressed in exocrine cells as a transcription factor. The depletion of SALL4 disrupts self-renewal and induces differentiation.

To clarify which of PDX1, PTF1A, or SALL4 determines the difference in Heinrich's classification, we examined the localization and number of positive cells. We analyzed the differential expression of PDX1, PTF1A, and SALL4 in large and small ducts in ectopic pancreas by immunohistochemistry. Results showed that the number of PTF1A-positive cells in large ducts was more widespread in type I than in type II in the gastro-duodenum, and more SALL4-positive cells were noticed in large ducts than in small ducts in the gastro-duodenum of type II. Our results revealed that PTF1A might promote exocrine differentiation in developing the pancreatic tissues, and that those with widespread expression differentiate into exocrine cells.

1. Introduction

Ectopic pancreas is defined as any isolated pancreatic tissue that the tissue except the pancreas and has no anatomical or vascular connection with normal pancreatic tissues [1]. Ectopic pancreas found in 0.6–13% of autopsies [2]. Belonging to congenital deformity, approximately 90% ectopic pancreases are located in the distal stomach (usually within 5 cm of the greater curvature of the pylorus), duodenum, and jejunum [1]. The treatment of ectopic pancreas varies with symptomatology, size, and potential malignancy. Management of pancreatic rest, especially at the gastroesophageal junction, including observation of conservative medical therapy, resection, or esophagectomy [3]. In recent years, the treatment of ectopic pancreas has shifted from open to laparoscopic surgery and more recently to robotic surgery [4].

The Heinrich classification is used to classify ectopic pancreas into three types. In type I, in which all components of the ducts, acini,

* Corresponding author.
E-mail addresses: s-renkei@adm.okayama-u.ac.jp (M. Chen), takehiro@md.okayama-u.ac.jp (T. Tanaka).

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and islets are observed. Type II consists of the acini and ducts. Type III consists only of ducts [5]. Tumors, such as ductal carcinoma, acinar cell carcinoma, or neuroendocrine tumor, can arise from ectopic pancreas. Patients with malignant transformation of ectopic pancreas are mostly middle-aged, and the stomach is the prime location. Most frequently, they are type I [6].

The site of organ or tissue formation is often determined by a developmental program of interactions between extracellular signals and intracellular networks of transcription factors. However, these developmental regulatory mechanisms sometimes do not function properly, and tissues occasionally develop ectopic sites in humans. However, the exact pathogenesis of ectopic pancreatic development remains unclear.

Identification of pancreas/duodenum homeobox protein 1 (PDX1) as a key regulatory transcription factor during embryonic development (distinguishing one body part from another) is central to developing transdifferentiation protocols. Ectopic expression factors like PDX1, Neurogenin 3 (Ngn3), and MafBZIP Transcription Factor A (MafA) which are vector-delivered transcription factors, induce reprogramming through extensive transcriptional remodeling [7].

PDX1 acts as a transcription factor in early pancreatic development, beta cell differentiation, and regulating insulin secretion expressed in beta cells of the adult pancreas and endocrine non-beta cells [8]. PDX1 is a highly efficient tool that redirects development of liver to the pancreas, and activation of PDX1 in transgenic tadpoles downregulate liver markers that converts the bulk of the liver to exocrine and endocrine pancreas [9]. The Hes1-mediated Notch pathway, that regulates the expression of pancreatic transcription factor 1 subunit alpha (PTF1A), is necessary for proper region specificity of the pancreas in the developing foregut endoderm in mice [10].

PTF1A is involved in exocrine cell formation and regulation of acinar cell identity, and is expressed in exocrine cells as a transcription factor [11]. PDX1 and PTFA1 are important factors in the differentiation of pancreatic progenitor cells on pancreatic organogenesis [12].

Sal-like protein 4 (SALL4), a factor of embryonic stem cells, plays an essential role in embryogenesis and oncogenesis. SALL4 expressed in extraembryonic endoderm cells and depletion of SALL4 disrupts self-renewal and induces differentiation. It regulates two distinct core circuits in embryonic cells and in extraembryonic endoderm cells which two distinct blastocyst-derived stem cell lines [13]. As reported for the proximal (PBGs)-to-distal (PDGs) maturational lineages, starting near the duodenum with cells expressing self-replication marker (SALL4) transitioning to PDG cells with no expression of pluripotency or self-replication markers and maintenance of pancreatic genes(PDX1), and expression of markers of pancreatic endocrine maturation [14]. We wonder to know if there is difference between the large duct and the small duct located in the stomach and duodenum (proximal) and jejunum (distal) of the ectopic pancreas with SALL4.

However, whether these transcription factors are involved in ectopic pancreas Heinrich type I and type II is unclear. In this study, we analyzed the differential expression of PDX1, PTF1A, and SALL4 in large and small ducts in ectopic pancreas by immunohistochemistry to determine whether these transcription factors differ between type I and type II in the large and small ducts of the ectopic pancreas. This study provides new insights into whether these transcription factors promote endocrine differentiation during ectopic pancreatic tissue development.

2. Material and methods

2.1. Patients

A series of (2002–2020) 23 ectopic pancreas cases diagnosed as Heinrich type I or type II were retrieved from formalin-fixed and paraffin-embedded (FFPE) archives of the Okayama University Hospital (Okayama, Japan). No cases of Heinrich type III were found.

Clinicopathological characteristics of the sample cohort and molecular analyses conducted in this study are reported in Table 1.

This study was approved by the Institutional Review Board of the Okayama University, Japan (2301-007). All clinical investigations were conducted in line with the principles of the Declaration of Helsinki.

2.2. Histological analysis

The FFPE blocks were sliced at a thickness of 4 μm, and stained with hematoxylin and eosin (H&E). Diagnosis was performed by two pathologists (Takehiro Tanaka and Takuro Igawa) who were blinded to the experimental design. Ectopic pancreas was classified according to the Heinrich classification system. In type I, all components of the ducts, acini, and islets were observed. Type II consisted of the acini and ducts [5].

Table 1
Patient clinical information of ectopic pancreas cohort.

Characteristics	
Age	24-82 (Median 52)
Gender	Female (9)
	Male (14)
Heinrich type	I (13)
	II (10)
Location	Stomach and duodenum (14)
	Jejunum (9)

Histologically, pancreatic ducts are divided into five portions: centroacinar, intercalated, intralobular, small interlobular, and main ducts. Centroacinar cells are terminal end duct cells that interface with acini. The terminal ducts or intercalated ducts are composed of flat epithelia and merge into intralobular ducts lined by cuboidal epithelia and merge to form small interlobular ducts surrounded by the mesenchyme. Larger interlobular ducts are lined by columnar epithelia [15].

Ectopic pancreatic ducts are divided into large and small ducts, which correspond to the main ducts and small interlobular ducts in the normal pancreas, respectively. According to H&E staining, the large ducts are usually composed of more than 100 cells, the perimeter is $>$ 100 μm, whereas those for small ducts are $<$100 cells and 100 μm.

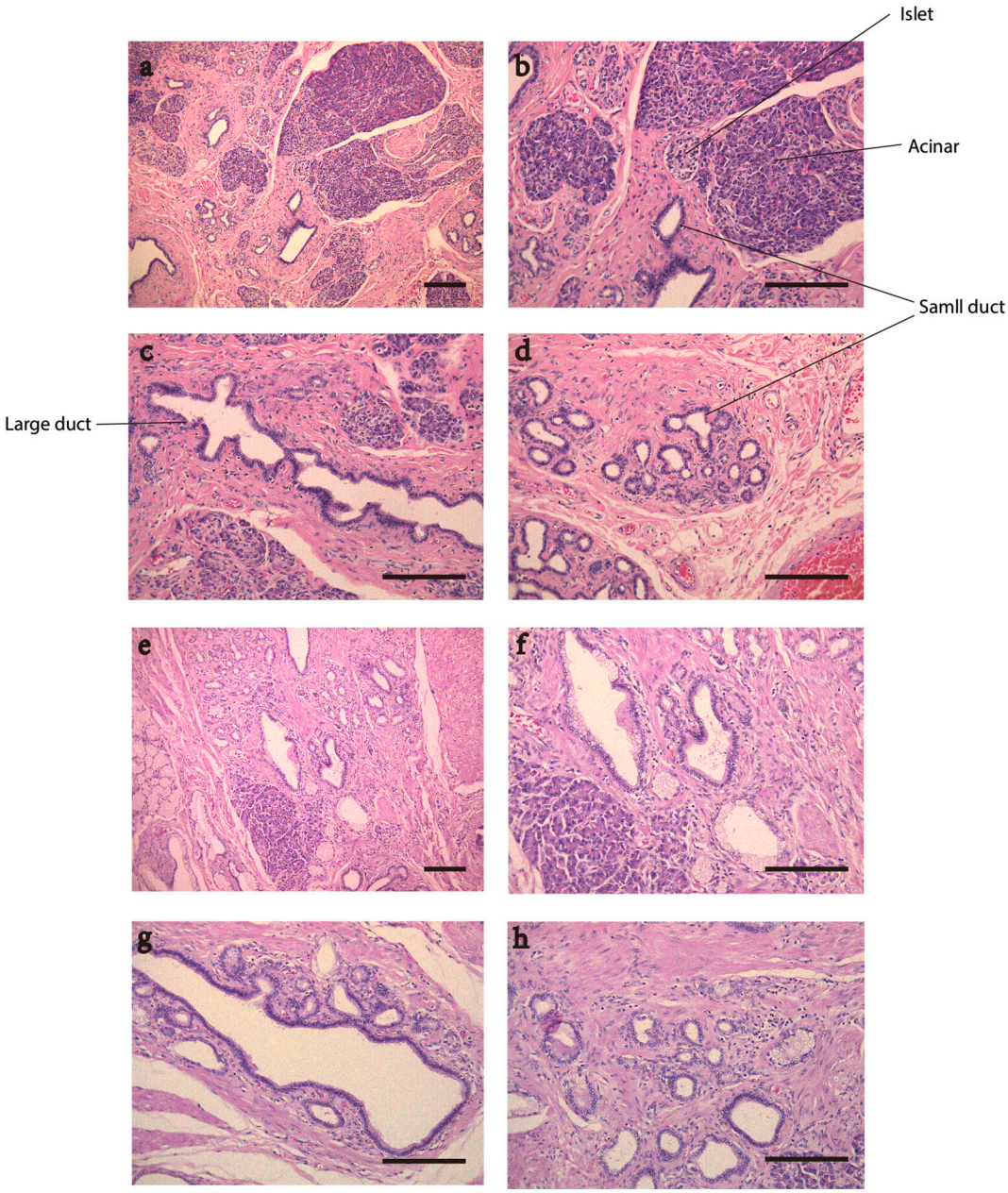

Fig. 1. H&E staining of Heinrich types I and II. The duct of type I located in the jejunum (a, 20 × magnification). The duct of type I (b, 40 × magnification). Large duct of type I (c, 40 × magnification). Small duct of type I (d, 40 × magnification). The duct of type II located in the duodenum (e, 20 × magnification). The duct of type II (f, 40 × magnification). Large duct of type II (g, 40 × magnification). Small duct of type II (h, 40 × magnification). Scale bar, 100 μm.

2.3. mmunohistochemistry (IHC)

FFPE sections (4 μm) from tissue blocks containing representative core samples were stained for immunohistochemical analysis and observed using a Leica Microsystems Bond-Max Autostainer System.

The following primary antibodies were used: *anti*-PDX1(1/500, rabbit monoclonal, clone EPR3358(2), ab134150; Abcam, Cambridge, UK), *anti*-SALL4 (1/100, mouse monoclonal, clone 6E3, ab57577; Abcam), and *anti*-PTF1A (1/200; mouse monoclonal, clone 1A2, H00256297-M05; Abnova, Taipei, Taiwan). Not all cases were stained for all markers owing to limitations in specimen size and block availability.

The number of positive cells and staining intensity scores were evaluated using an open software for positive cell detection in QuPath 0.3.2 (https://qupath.github.io, accessed on December 15, 2022). For each case, the chromogenic stain is observed on the left side and stain with cell detection on the right side. QuPath detects negative or positive cells as blue or red (Fig. 2).

2.4. Statistical analyses

Statistical analyses were performed using GraphPad Prism (version 9, San Diego, CA, USA). To assess the difference between types I and II, we used an unpaired *t*-test for quantitative variables. p-value <0.05 was considered statistically significant.

3. Results

To independently validate the prognostic potential of PDX1, PTF1A, and SALL4, we analyzed data from 23 patients containing eight females in the ectopic pancreas cohort (Table 1). Thirteen patients showed Heinrich I stomach and duodenum (8) and jejunum (5). Ten patients showed Heinrich II stomach and duodenum (6) and jejunum (4).

H&E staining showed the duct, acini, and islets in the type I, which were located in the jejunum (Fig. 1a–d). In the type II, ducts and acini were located in the duodenum (Fig. 1e–h). No significant difference in age or sex was observed between types I and II ($p = 0.2103$ and 0.5, respectively).

3.1. Expression and localization of PDX1

IHC analysis showed widespread PDX1 expression in the large and small ducts of Heinrich types I and II in most patients. No significant difference was observed in PDX1 expression between the large and small ducts of Heinrich types I and II, which located in the gastro-duodenum and jejunum (Fig. 3a, d; 4a, d, g).

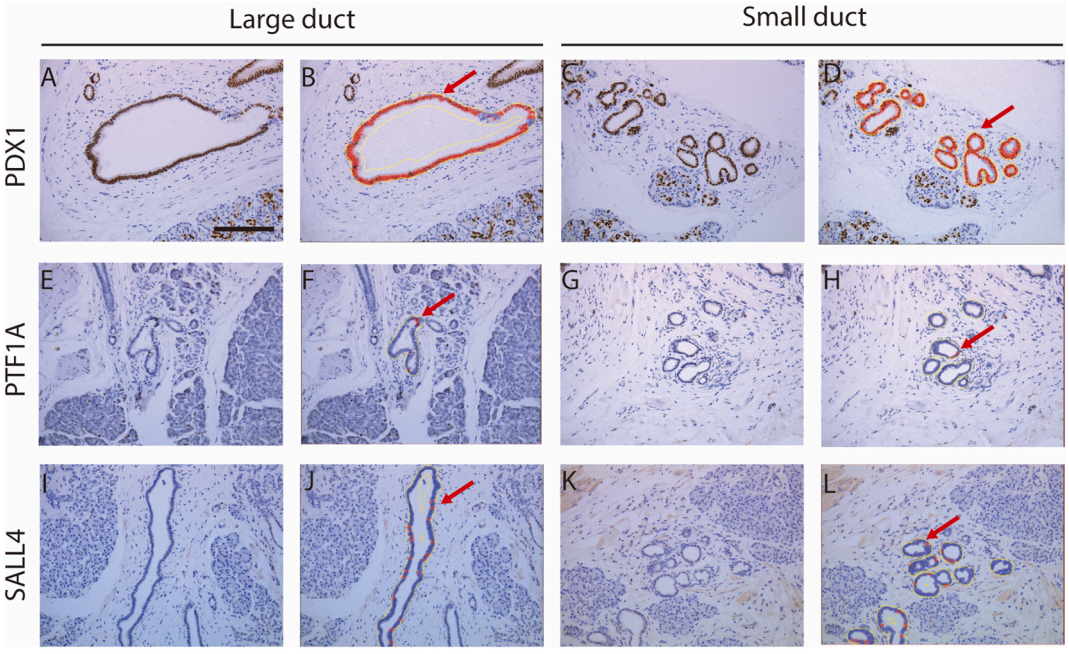

Fig. 2. Immunohistochemical analysis of expression of PDX1, PTF1A, and SALL4 in ectopic pancreas using QuPath automated image analysis (40 × magnification). Representative images showing an original core (left) and QuPath markup image (right) are presented. Scale bar, 100 μm.

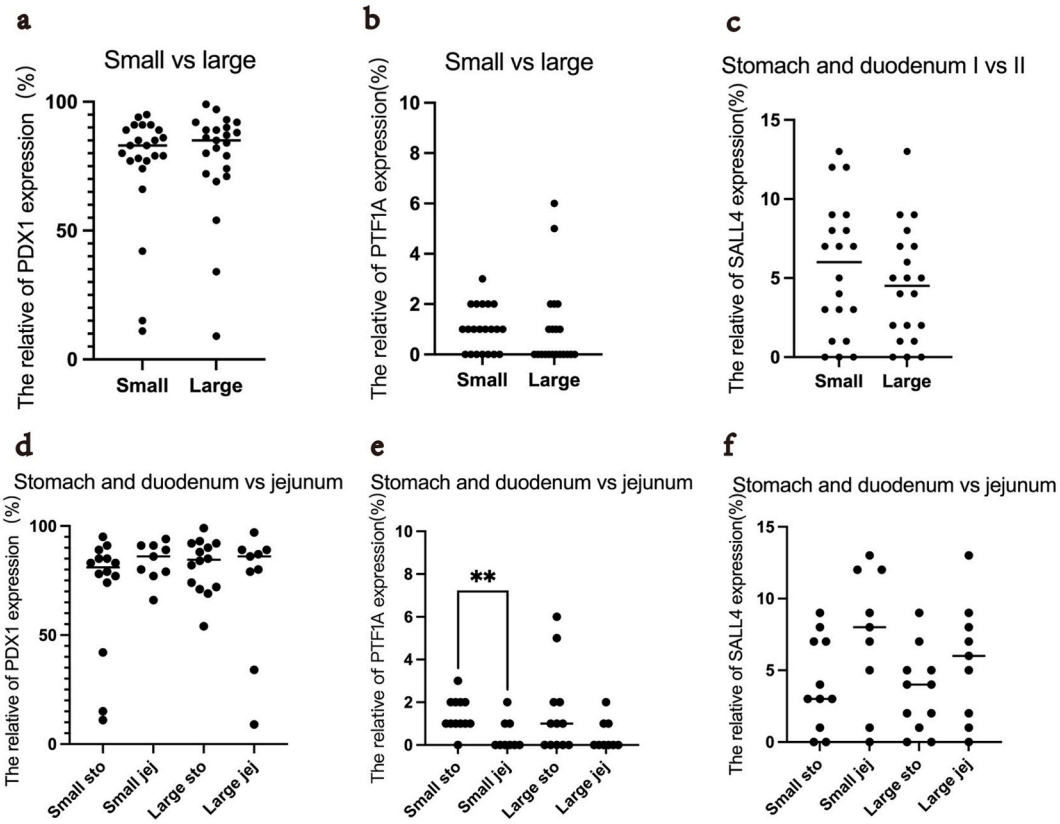

Fig. 3. Dot plots for proportions of cells positive for PDX1, PTF1A, and SALL4 from immunohistochemical expression analysis (without Heinrich classification).

3.2. Expression and localization of PTFA1

In the IHC analysis of PTF1A expression, a significantly large number of type I-positive cells was found in the large duct of the stomach and duodenum ($p = 0.0382$) (Fig. 4b). No significant difference was noticed between the small duct of the stomach and duodenum in types I and II ($p = 0.549$) (Fig. 4b). Moreover, no significant difference was noticed between the large and small ducts of the jejunum in types I and II ($p = 0.1176$, 0.2874, respectively) (Fig. 4e). In type I, PTF1A expression in the stomach and duodenum was more widespread than that in the jejunum ($p = 0.002$) (Fig. 4h). No significant difference was observed in type II ($p = 0.8446$) (Fig. 4h). Regarding the small duct, PTFA1 expression in stomach and duodenum was more widespread than that in the jejunum ($p = 0.0096$) (Fig. 3e).

3.3. Expression and localization of SALL4

IHC analysis of SALL4 expression showed that the number of patients having small duct from type II was significantly more widespread than those having small duct from type I of the stomach and duodenum ($p = 0.038$) (Fig. 4c). No significant difference was noticed between the large duct of the stomach and duodenum in types I and II ($p = 0.657$) (Fig. 4c). Moreover, no significant difference was noticed between the large and small ducts of the jejunum in types I and II ($p = 0.8467$, 0.8192) (Fig. 4f).

As shown in Fig. 4i, SALL4 expression in the stomach and duodenum was rare than that in the jejunum in type I ($p = 0.0291$). No significant difference was observed in type II ($p = 0.4126$) (Fig. 4i). No significant difference was observed in SALL4 expression between the large and small ducts of Heinrich types I and II, which located in the gastro-duodenum and jejunum (Fig. 3c and f).

4. Discussion

In the present study, we performed IHC analyses to elucidate the transcription factors PDX1, PTF1A, and SALL4 that affect ectopic pancreas located in the gastro-duodenum and jejunum between Heinrich types I and II and constructed dot plots to analyze the proportions of cells positive for PDX1, PTF1A, and SALL4.

No significant difference in PDX1 expression was observed between the gastro-duodenum and jejunum, suggesting that PDX1 expression does not define the type of ectopic pancreas.

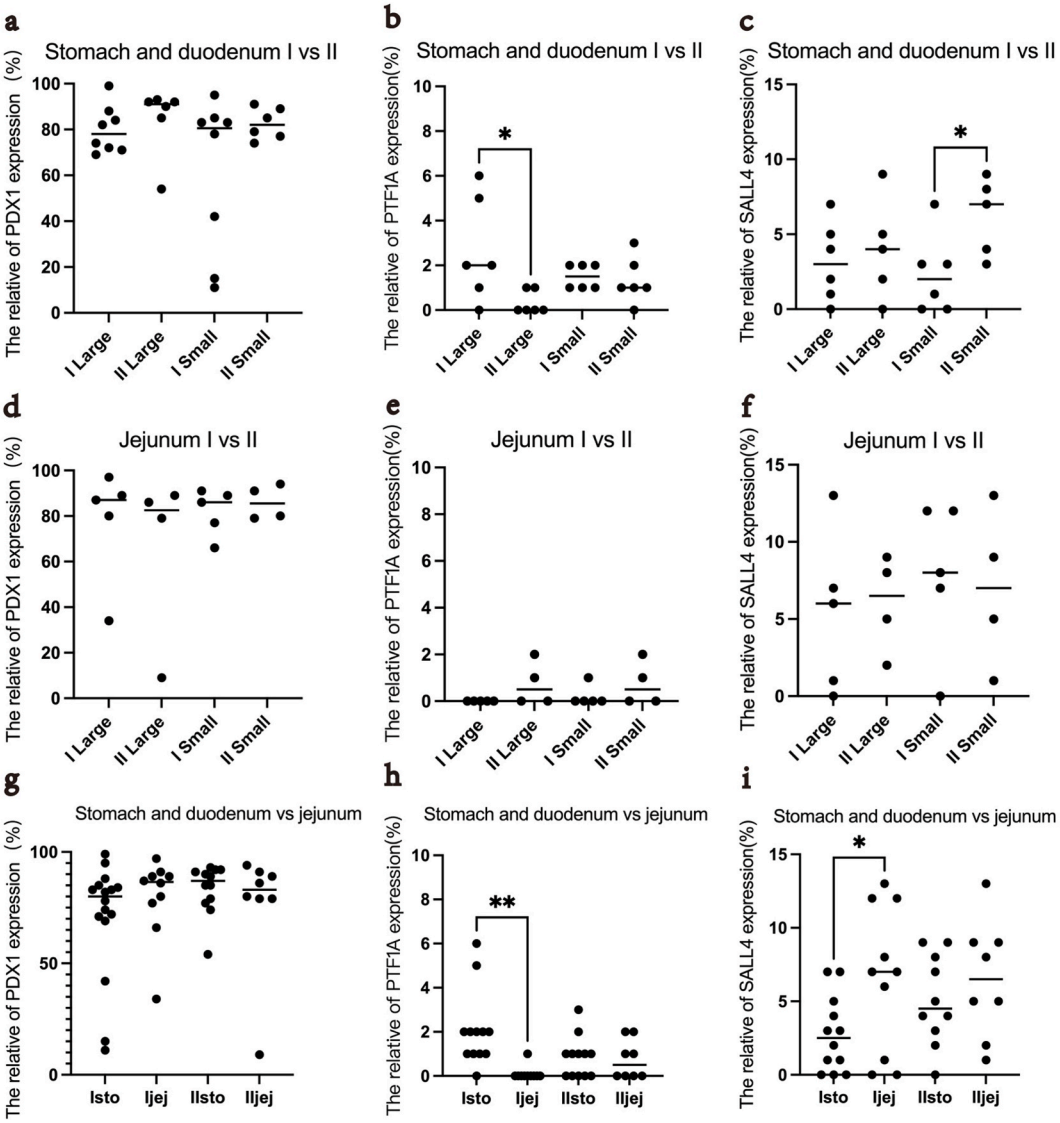

Fig. 4. Dot plots for proportions of cells positive for PDX1, PTF1A, and SALL4 from immunohistochemical expression analysis (within Heinrich classification).

In the gastro-duodenum, the number of PTF1A-positive cells in large ducts was more widespread expression in type I than in type II, and no significant difference in their numbers was observed in small ducts (Fig. 4b). A study using mouse embryonic stem cells with tetracycline-inducible expression of PTF1A *in vitro* revealed that PTF1A-induced cultures differentiated into significantly more endocrine and exocrine cells [16], and those with rare expression differentiated into exocrine cells; therefore a downregulation of PTF1A may regulate the exocrine-to-endocrine switch in the mature pancreas [17]. Our results suggest that PTF1A might promote exocrine differentiation in the development of pancreatic tissue, those with widespread expression differentiate into exocrine cells. PTF1A may be involved in ectopic pancreatic differentiation in small duct and type I in stomach and duodenum respectively, compared with jejunum.

We observed more SALL4-positive cells in type II than type I in the small duct of the gastro-duodenum. Interestingly, the number of stem cells was more widespread in type II, which is composed of fewer components than in type I; this result was opposite to that of PTF1A expression. However, this interesting result requires further verification. There is no significant difference between the stomach and duodenum (proximal) and jejunum (distal) of the ectopic pancreas with SALL4. This suggests that sall4 may not be involved in the proximal-to-distal transition.

In summary, our results revealed that PTF1A might promote exocrine differentiation during the development of gastroduodenal pancreatic tissue, and the mechanisms of ectopic pancreatic development should be investigated in future studies. Further mining of this valuable resource might reveal the mechanisms of pancreatic development, neighboring tissue interactions, and pathological

mechanisms of ectopic pancreas to help in developing new treatment strategies.

Author contribution statement

Mengxi Chen: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Takehiro Tanaka: Conceived and designed the experiments.
Takuro Igawa, Tadashi Yoshino: Analyzed and interpreted the data.
Yanyan Han, Fangli Peng: Contributed reagents, materials, analysis tools or data.
Zaishun Jin: Analyzed and interpreted the data; Wrote the paper.
Tadashi Yoshino: Analyzed and interpreted the data.

Data availability statement

The authors do not have permission to share data.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Takehiro Tanaka has patent licensed to 2301-007.

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