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ORIGINAL ARTICLE

Hormonally defined pancreatic and duodenal neuroendocrine tumors differ in their transcription factor signatures: expression of ISL1, PDX1, NGN3, and CDX2

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Abstract We recently identified the transcription factor (TF) islet 1 gene product (ISL1) as a marker for well-differentiated pancreatic neuroendocrine tumors (P-NETs). In order to better understand the expression of the four TFs, ISL1, pancreatico-duodenal homeobox 1 gene product (PDX1), neurogenin 3 gene product (NGN3), and CDX-2 homeobox gene product (CDX2), that mainly govern the development and differentiation of the pancreas and duodenum, we studied their expression in hormonally defined P-NETs and duodenal (D-) NETs. Thirty-six P-NETs and 14 D-NETs were immunostained with antibodies against the four pancreatic hormones, gastrin, serotonin, calcitonin, ISL1, PDX1, NGN3, and CDX2. The TF expression pattern of each case was correlated with the tumor's hormonal profile. Insulin-positive NETs expressed only ISL1 (10/10) and PDX1 (9/10). Glucagon-positive tumors expressed ISL1 (7/7) and were almost negative for the other TFs. Gastrin-positive NETs, whether of duodenal or pancreatic origin, frequently expressed PDX1 (17/18), ISL1 (14/18), and NGN3 (14/18). CDX2 was mainly found in the gastrin-positive P-NETs (5/8) and rarely in the D-NETs (1/10). Somatostatin-positive NETs, whether duodenal or pancreatic in origin, expressed ISL1 (9/9), PDX1 (3/9), and NGN3 (3/9). The remaining tumors showed labeling for ISL1 in addition to NGN3. There was no association between a particular TF pattern and NET features such as grade, size, location, presence of metastases, and functional activity. We conclude from our data that there is a correlation between TF expression patterns and certain hormonally defined P-NET and D-NET types, suggesting that most of the tumor types originate from embryologically determined precursor cells. The observed TF signatures do not allow us to distinguish P-NETs from D-NETs.

Keywords Neuroendocrine tumors · Pancreas · Duodenum · Insulinoma · Glucagonoma · Gastrinoma · Transcription factors · ISL1 · PDX1 · NGN3 · CDX2

Abbreviations

ISL1	Islet 1 gene product
PDX1	Pancreatico-duodenal homeobox 1 gene product
NGN3	Neurogenin 3 gene product
CDX2	CDX-2 homeobox gene product

Introduction

Transcription factors (TFs) that play a crucial role in the early development of endocrine cells in the intestine and the pancreas are CDX-2 homeobox gene product (CDX2), pancreatico-duodenal homeobox 1 gene product (PDX1), islet 1 gene product (ISL1), and neurogenin 3 gene product

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(NGN3). CDX2 governs the differentiation of the intestinal cells into enterocytes, Paneth cells, goblet cells, and endocrine cells. In adult life, it is expressed in terminally differentiated intestinal cells [1]. In endocrine cells, nuclear CDX2 immunoreactivity was found in all serotonin-producing cells, in about 10% of gastrin-producing cells, in about 30% of gastric inhibitory peptide cells, and in a few motilin-positive cells of the normal intestinal mucosa, while other gastrointestinal endocrine cell types were CDX2 negative [1–3]. PDX1 directs the development of both the exocrine and the endocrine pancreas [4, 5]. During adult life, PDX1 is expressed in islet cells, especially insulin cells, and some centro-acinar and ductal cells [6]. NGN3 is a transcription factor that is responsible for endocrine differentiation of intestinal progenitor cells, temporarily directs the development of the four endocrine cell lineages in the pancreas, and is expressed in somatostatin and gastrin cells of the duodenum [5, 7–9]. ISL1 is a transcription factor that binds to an islet beta cell-specific enhancer element in the insulin gene. In adult life, it is expressed in all pancreatic endocrine cell types [10–12].

Because neoplasms in general and neuroendocrine tumors in particular recapitulate embryological–cellular differentiation, it is of interest to analyze the expression of TFs in NETs, first in order to trace the cell lineage of these tumors back to their origin [13], and second to study the association between the expression of TFs and the biology of the neoplasms. So far, most investigations focused on the expression of CDX2 and found that this TF is a hallmark of neoplasms derived from the intestine, including NETs [1, 2, 14]. Almost all NETs of the ileum and appendix (ileal and appendiceal carcinoids) were found to be CDX2 positive [1], while only a minority of pancreatic and other extraintestinal NETs showed CDX2 expression [1]. ISL1 expression, on the other hand, characterized pancreatic NETs and their metastases [15]. PDX1 was found to be expressed in almost 40% of pancreatic endocrine neoplasms [6]. In another study, PDX1 labeled pancreatic but not duodenal gastrinomas [16]. As for NGN3, data on its expression were reported in individual hormonally defined pancreatic NETs, which often showed combined nuclear–cytoplasmic staining [17].

Pancreatic and duodenal NETs are very much interrelated. Gastrin- and somatostatin-producing NETs may occur in both sites. Moreover, both groups of NETs show conspicuous heterogeneity in terms of clinical syndromes and biology, as defined by the hormonal cell type that composes the neoplasm [18]. The origin of these hormonally characterized neoplasms may be a common stem cell that is capable of giving rise to all the different hormone-producing cells encountered. Alternatively, the tumor originates from preprogrammed cells that give rise to cells committed to a certain cell lineage.

The objective of this study was to analyze the expression of ISL1, PDX1, NGN3, and CDX2 in hormonally defined pancreatic and duodenal NETs in order to find out whether these four key TFs yield signatures that characterize certain tumor entities and indicate a pancreatic or duodenal origin of the tumors. To this end, we examined a series of well-differentiated pancreatic and duodenal NETs producing either insulin, glucagon, somatostatin, pancreatic polypeptide, gastrin, or calcitonin as the predominant hormone. These NETs included tumors with and without a hormonal syndrome and with either benign or malignant behavior. The results revealed that hormonally defined NETs of the pancreas and duodenum may display distinct TF signatures.

Material and methods

A total of 50 resected NETs of the duodenum and pancreas that had been collected between 1984 and 2009 were retrieved from the consultation archives of the Departments of Pathology of the Technical University München and the University of Kiel (Germany). A number of these tumor cases were included in previous studies investigating different aspects of duodenal and pancreatic NET biology. The resection procedures included tumor excision, enucleation, duodenectomy, Whipple operations, and left partial pancreatectomy. The tumor series comprised 50 primary NETs, 14 from the duodenum (bulb, second part, some of them periampullary, and one from the distal part), and 36 from the pancreas.

The tumors were classified according to recent WHO criteria for endocrine tumors [19]. All NETs were well differentiated and therefore designated as G1 (54%) or G2 (46%) NETs, regardless of whether they showed evidence of malignant behavior or not. They were grouped on the basis of their predominant (more than 60–80%) immunohistochemical hormone expression and hormonal syndrome. Classified according to the previous WHO classification [20, 21], there were 13 well-differentiated endocrine tumors and 37 well-differentiated endocrine carcinomas.

The following tumor groups were defined: ten insulin-producing P-NETs (all insulinomas); seven glucagon-producing P-NETs (four glucagonomas and three nonfunctioning tumors); ten nonfunctioning somatostatin-producing NETs (five of pancreatic and five of duodenal origin); three nonfunctioning polypeptide-producing P-NETs; 18 gastrin-producing NETs (including five gastrinomas, five nonfunctioning tumors of duodenal origin, and eight gastrinomas of pancreatic origin), one nonfunctioning calcitonin-expressing P-NET, and two nonfunctioning P-NETs without significant hormone expression.

All tissue blocks were formalin fixed and paraffin embedded. Appropriate hematoxylin and eosin-stained sections, most of which contained tumor tissue next to normal parenchyma, were examined. Immunostaining was performed on 3-μm-thick sections. They were immunostained for synaptophysin (polyclonal antibody, DakoCytomation, Glostrup, Denmark, 1:50), chromogranin A (monoclonal antibody, Ventana medical systems, Tucson, AZ, USA, 1:2), insulin (monoclonal antibody, Biogenex, San Ramon, CA, USA, 1:40), glucagon (polyclonal antibody, Biogenex, 1:60), somatostatin (polyclonal antibody, DakoCytomation, 1:200), pancreatic polypeptide (PP, polyclonal antibody, DakoCytomation, 1:5000), gastrin (polyclonal, Paesel, Frankfurt, Germany, 1:3000), PDX1 (1:200, clone 267712, R&D Systems, Wiesbaden, Germany), ISL1 (rat 1:100, C-terminal portion, Developmental Studies Hybridoma Bank, Univ. of Iowa, Iowa City, USA), NGN3 (neurogenin 3, 1:500, Developmental Studies Hybridoma Bank, University of Iowa), and CDX2 (1:20, clone AMT28, Novocastra Laboratories Ltd, Newcastle, UK). MIB1 staining was performed using a mouse monoclonal antibody (M0722, DAKO, Hamburg, Germany 1:100). Immunostaining was carried out using the avidin–biotin peroxidase complex method. All immunohistochemical staining was performed after high temperature antigen retrieval (125°C for 1 min) using Dako Pascal Microprocessor/heat-induced epitope retrieval procedures at pH 6.7. Endogenous peroxidase blocking was performed for 15 min before application of the primary antibody. Positive internal controls included normal duodenal mucosa for CDX2, normal pancreas for ISL1 and PDX1, and isolated endocrine cells of the duodenal and submucosal and intrapancreatic nerve fibers for NGN3. Negative controls consisted in omitting the primary antibody and replacing it with normal rabbit serum at an equivalent concentration. Positive staining for the TFs was visualized as a dark brown reaction localized to the nucleus. It was considered strong if it had the same intensity as the internal control, weak if it was barely visible without a cytoplasmic stain, and moderate if it was weaker than that of the internal control tissue but still well recognizable. Negative immunostaining was recorded if only a few isolated cells were weakly or moderately positive (<5%). Positive immunostaining was recorded and scored as 1 if 5–10% of the cells were positive; 2 if 10–50% of the cells were positive, and 3 if more than 50% of the cells were positive. In cases with strong nuclear positivity, the percentage of stained cells always exceeded 5%. The results for Mib1 were given as percentage of positive tumor cells.

Results

The study included 50 NETs of the pancreas (36/50, 71%) and the duodenum (14/50, 29%), whose detailed clinicopathological data are listed in Table 1. There were 24 female (48%) and 26 male patients (52%) with ages ranging from 17 to 88 years. The size of the tumors ranged from 0.6 to 14 cm. A hormonal syndrome was observed in 54% of the cases. Seventy-two percent of the pancreatic NETs and 70% of the duodenal NETs were classified as tumors associated with malignant behavior.

All NETs stained for synaptophysin and chromogranin A. Table 2 summarizes the immunohistochemical findings for the seven hormones and the four TFs tested. Immunostaining for the hormones revealed a monohormonal pattern (i.e., a significant expression of only one hormone) in 9/10 insulin-positive NETs, in 6/7 glucagon-positive NETs, in

Table 1 Clinicopathologic features of pancreatic and duodenal neuroendocrine tumors (NETs)

NET type[a]	No.	Age (mean, years)	Sex (m/f)	Site	Size (mean, cm)	Hormonal syndrome[b]	WHO		Grade		Met[c]
							wdet	wdec	1	2	
INS expressing	10	51.4	6/4	Pancreas	1.9	10/10	6/10	4/10	8/10	2/10	1/10
GLU expressing	7	57.4	3/4	Pancreas	6.2	4/7	1/7	6/7	2/7	5/7	5/7
SOM expressing	5	46	1/4	Pancreas	6.4	0/5	1/5	4/5	3/5	2/5	1/5
	4	58.5	3/1	Duodenum	3.8	0/4	0/4	4/4	1/4	3/4	2/4
PP expressing	3	57.7	0/3	Pancreas	6.4	0/3	1/3	2/3	0/3	3/3	1/3
CAL/non-expressing	3	65	1/2	Pancreas	6	0/3	0/3	3/3	0/3	3/3	0/3
GAS expressing	8	53	5/3	Pancreas	4.9	8/8	0/8	8/8	5/8	3/8	5/8
	10	71	7/3	Duodenum	1.4	5/10	4/10	6/10	8/10	2/10	3/10

INS insulin, *GLU* glucagon, *SOM* somatostatin, *PP* pancreatic polypeptide, *CAL* calcitonin, *GAS* gastrin, *wdet* well-differentiated endocrine tumors, *wdec* well-differentiated endocrine carcinomas, *NETs* neuroendocrine tumors, *MET* metastases, *m* male, *f* female

[a] Predominant hormone expression

[b] Hypoglycemic hyperinsulinism, glucagonoma syndrome or Zollinger-Ellison-syndrome

[c] Metastases, lymph node and/or liver

Table 2 Expression of the transcription factors PDX1, ISL1, NGN3, and CDX2 in pancreatic and duodenal NETs

NET type[a]	Site	Transcription factors				Other hormone expression
		PDX1	ISL1	NGN3	CDX2	
INS expressing	Pancreas	9/10 (1/0/8)[b]	10/10 (2/0/8)	0/10	0/10	SOM1/10
GLU expressing	Pancreas	1/7 (1/0/0)	7/7 (1/0/6)	0/7	1/7 (0/1/0)	PP 1/7
SOM expressing	Pancreas	1/5 (0/0/1)	5/5 (0/1/4)	2/5 (1/0/1)	0/5	PP 1/5
	Duodenum	2/4 (0/0/2)	4/4 (0/0/4)	1/4 (0/1/0)	0/4	0/4
PP expressing	Pancreas	0/3	3/3 (0/0/3)	1/3 (0/1/0)	0/3	0/3
CAL/non-expressing	Pancreas	0/3	3/3 (0/0/3)	1/3 (0/0/1)	0/3	GLU 1/3
GAS expressing	Pancreas	7/8 (3/3/1)	4/8 (1/2/1)	5/8 (2/2/1)	5/8 (2/2/1)	PP 2/8
	Duodenum	10/10 (2/0/8)	10/10 (1/1/8)	9/10 (2/2/5)	1/10 (0/0/3)	SERO 1/10

INS insulin, *GLU* glucagon, *SOM* somatostatin, *PP* pancreatic polypeptide, *CAL* calcitonin, *GAS* gastrin, *SERO* serotonin, *NET* neuroendocrine tumor, *ISL1* islet 1 gene product, *PDX1* pancreatico-duodenal homeobox 1 gene product, *NGN3* neurogenin 3 gene product, *CDX2* CDX-2 homeobox gene product

[a] Predominant hormone expression

[b] No. of cases with 5–10%, 10–50%, or >50% positive cells

5/6 PP- and calcitonin-positive NETs, in 5/18 gastrin-positive NETs, and in 8/9 somatostatin-positive NETs.

TF patterns by tumor type

All insulin-positive NETs expressed ISL1 and 9/10 expressed PDX1 (Table 2 and Fig. 1). In contrast, none of the insulin-positive NETs stained for CDX2 or NGN3. All glucagon-positive tumors expressed ISL1 (Fig. 2). In addition, there was weak staining for PDX1 and CDX2 in two different glucagon-positive NETs. The PP-positive tumors, the calcitonin-positive tumor and the non-expressing P-NETs all labeled for ISL1 and 2/6 additionally for NGN3, while they were negative for PDX1 and CDX2. Gastrin-positive NETs, whether of duodenal or pancreatic origin, frequently expressed PDX1 (17/18), ISL1 (14/18), and NGN3 (14/18) (Fig. 3). CDX2 was mainly found in the gastrin-positive P-NETs (5/8) and rarely in the D-NETs (1/10). All somatostatin-positive NETs, whether duodenal or pancreatic in origin, expressed ISL1, and 3/9 expressed PDX1. NGN3 was also expressed by 3/9 (Fig. 2).

TF expression frequency

ISL1 was expressed in all NETs of this series, except for four gastrin-positive P-NETs (92%). PDX1 was predominantly found in insulin- and gastrin-positive NETs (26/28, 92%). In addition to PDX1 and ISL1, NGN3 was commonly expressed in gastrin-positive P-NETs and D-NETs (14/18, 77%), while it was rarely found in other NETs (6/32, 2%). CDX2 was only found in five P-NETs (one glucagon and four gastrin positive) and one D-NET (6/50, 12%).

TF expression and biological features

The biological NET features such as location, grade, size, presence of metastases, and functional activity did not correlate with any particular TF pattern (Table 3).

Discussion

The expression of TFs has successfully been used to identify the site of origin of neoplasms. CDX2, for instance, serves as a marker for intestinal type carcinomas, including NETs of the ileum and the appendix [1, 2], while ISL1 was found to label predominantly P-NETs [15]. This study, which is restricted to the expression of ISL1, PDX1, NGN3, and CDX2 and based on a functionally well defined, though rather small, number of NETs from the pancreas and duodenum, revealed that the expression patterns of the tested TFs show a relationship to hormonally defined P- and D-NET types such as insulinomas, glucagonomas, gastrinomas, and others. The four TFs failed, however, to distinguish whether gastrin- or somatostatin-positive NETs were of duodenal or pancreatic origin.

Insulin-producing tumors form a rather homogeneous group among the P-NETs [22]. The majority of them are smaller than 2 cm, behave benignly after resection, are monohormonal (i.e., rarely contain other endocrine cells than insulin cells), occur in the pancreas and are functionally active so that they are all termed insulinomas. In our study, which included insulinomas considered to be benign or malignant, all expressed the TFs ISL1 and PDX1. This expression pattern closely reflects the expression of these

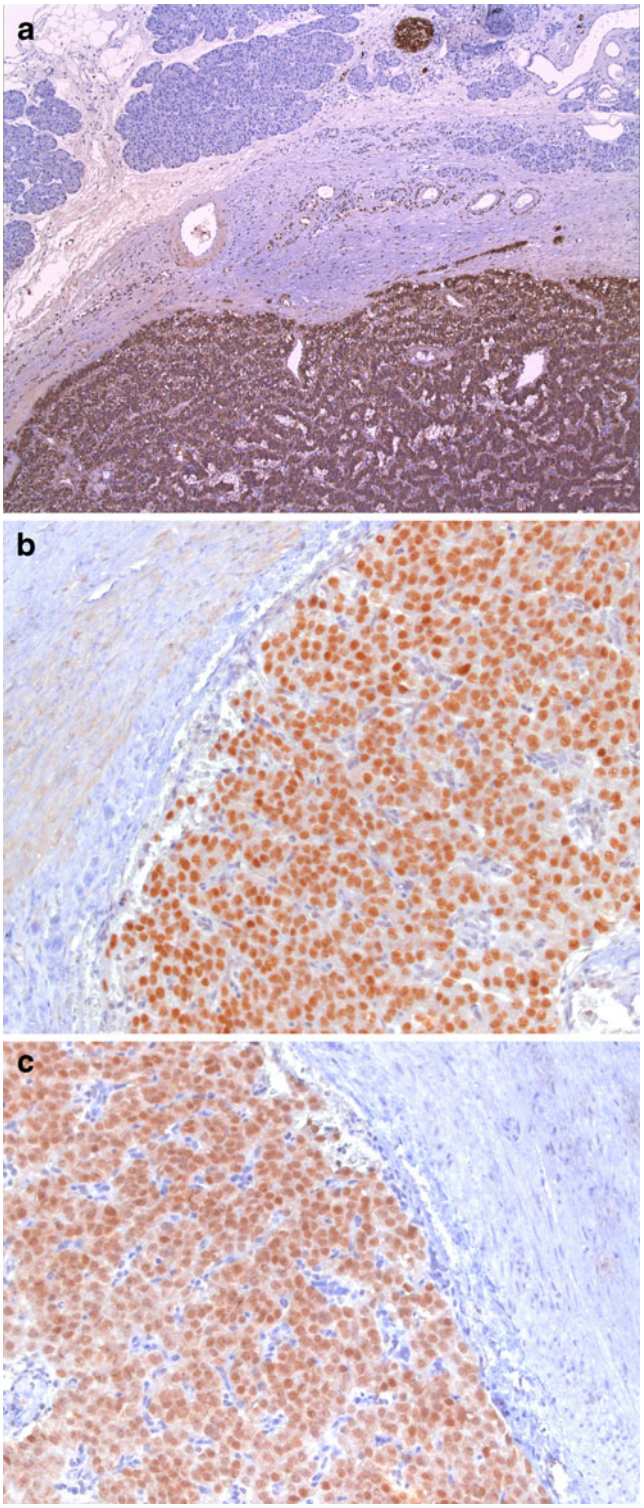

Fig. 1 Pancreatic well-differentiated tumor (insulinoma): immunostaining for insulin (**a**), ISL1(**b**), and PDX1 (**c**)

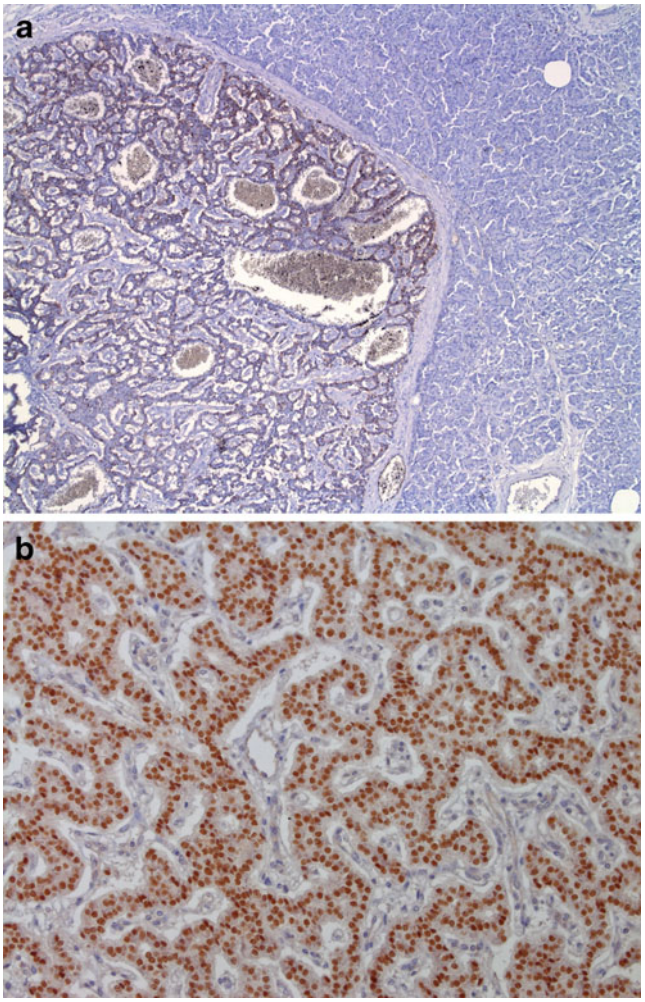

Fig. 2 Pancreatic well-differentiated tumor (glucagon producing): immunostaining for glucagon (**a**) and ISL1(**b**)

TFs in the normal pancreas, where ISL1 is exclusively and PDX1 predominantly expressed in the nuclei of islet cells. In the case of PDX1, the islet cell labeling resides in the nuclei of the beta cells, while ISL1 occurs in all islet cell types. The strong expression of ISL1 and PDX1 in insulinomas combined with a complete absence of NGN3 and CDX2 was not observed in any of the other P-NETs or D-NETs tested. It is thus highly characteristic for this specific type of P-NET.

NETs positive for glucagon may be functionally active, in which case they are called glucagonoma, or inactive and are then termed glucagon-producing tumor [19, 21, 23]. They occur predominantly in the pancreas and are frequently malignant when sporadic and larger than 2 cm. When associated with MEN1, they are benign and often cystic [24]. Here, we found that all glucagon-positive tumors, regardless of their biological features, strongly expressed ISL1 and were (with two exceptions) almost completely negative for the other three TFs tested. This TF signature is, like that of the insulinomas, unique among the various types of P-NET and D-NET in our series. It reflects the fact that the mature glucagon cell in the pancreas is solely ISL1-positive and does not express either PDX1,

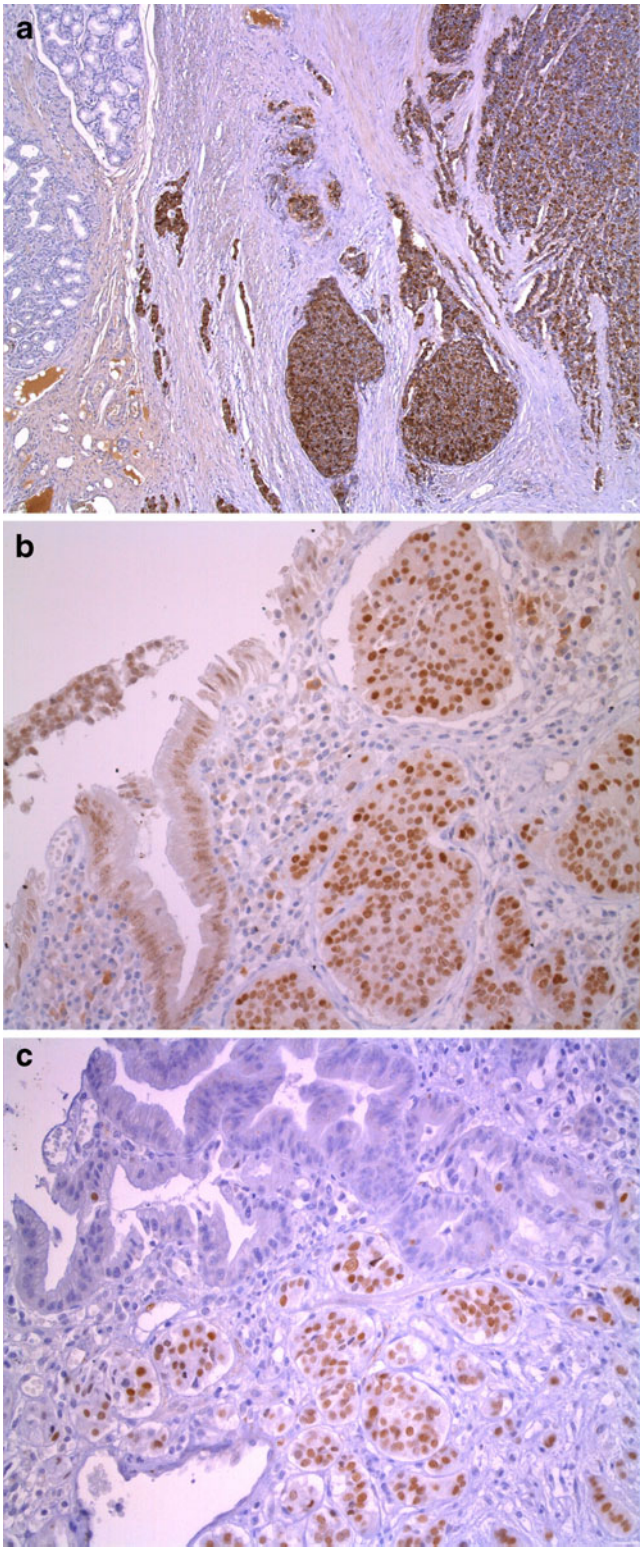

Fig. 3 Duodenal well-differentiated neuroendocrine tumor (gastrinoma): immunostaining for gastrin (**a**), PDX1 (**b**), and NGN3 (**c**)

NGN3, or CDX2. The neoplastic glucagon cell therefore appears to preserve the cell type specific features of the nonneoplastic cell lineage from which it might derive and thereby distinguishes itself from other cell lineages giving rise to P-NETs.

The remaining P-NET types, including PP- and calcitonin-producing and hormonally non-expressing tumors, show more complex TF expression patterns than insulinomas and glucagonomas. They all label for ISL1 and, in addition, variably for PDX1 and/or NGN3. Though the informative value of the TF patterns in this group is limited by the small number of tumors tested, it might indicate, on the one hand, that the cell lineages giving rise to these tumors differ from those associated with insulinomas and glucagonomas. On the other hand, it might suggest that ISL1, PDX1, and NGN3 may collaborate in the cell lineages forming these P-NETs.

A special group of tumors in our series is the one that includes NET types occurring in both the pancreas and the duodenum. These are the gastrin-producing and the somatostatin-producing NETs. While the former tumors included functionally active NETs, i.e., gastrinomas, and nonfunctioning gastrin-producing tumors (all of duodenal origin), the latter tumors were all nonfunctioning. Somatostatin-producing NETs, whether duodenal or pancreatic in origin, showed a TF pattern that was characterized by the expression of ISL1 together with occasional positivity for PDX1 and NGN3 in the absence of CDX2. Gastrin-producing NETs, whether functioning or not, whether of duodenal or pancreatic origin, presented in most cases with a "full house" TF signature consisting of the expression of ISL1, PDX1, NGN3, and CDX2.

Although the distinction between the expression patterns of the hormonally defined P-NETs and D-NETs in our series was not completely "black and white", they showed distinct differences that suggest an origin from different cell lineages. This is most obvious for the insulinomas and glucagonomas. However, it is also recognizable in other tumor entities tested. For instance, in the case of the gastrin-positive NETs it may be speculated that the gastrin-positive P-NETs and D-NETs originate from two different cell lineages. While the gastrin-positive P-NETs may be related to a cell lineage with intestinal features, since they commonly expressed the intestinal transcription factor CDX2 in addition to ISL1, PDX1, and NGN3, the gastrin-positive D-NETs almost entirely lacked CDX2 expression and may therefore have a cell origin distinct from that of their pancreatic counterparts. How the gastrin-producing tumors in the pancreas keep contact with the intestine is not known, but it is interesting to note that the pancreatic gastrinomas preferably occur in the head region of the gland, close to the duodenum. In the case of the somatostatin-producing NETs, the cell of origin appears to be pancreatic rather than intestinal in nature, as their TF signature lacks the intestinal marker CDX2. In this context, it is of interest that the duodenal somatostatin-producing

Table 3 Expression of the transcription factors PDX1, ISL1, NGN3, and CDX2 in pancreatic and duodenal NETs correlated with clinicopathologic features

NET type	Site	F/NF	Behavior	Transcription factors			
				PDX1	ISL1	NGN3	CDX2
INS expressing	Pancreas	F 10/10	ben 9/10	5/6	5/6	0/6	0/6
			mal 1/10	4/4	4/4	0/4	0/4
GLU expressing	Pancreas	F 4/7	mal 4/4	0/4	4/4	0/4	0/4
		NF 3/7	ben 1/3	0/1	1/1	0/1	0/1
			mal 2/3	1/2	2/2	0/2	1/2
SOM expressing	Pancreas	NF 5/5	ben 4/5	1/4	4/4	2/4	0/4
			mal 1/5	0/1	1/1	0/1	0/1
	Duodenum	NF 4/4	ben 2/4	1/2	2/2	1/2	0/2
			mal 2/4	1/2	2/2	0/2	0/2
PP expressing	Pancreas	NF 3/3	ben 2/3	0/2	2/2	1/2	0/2
			mal 1/3	0/1	1/1	0/1	0/1
CAL/non-expressing	Pancreas	NF 3/3	ben 2/3	0/2	2/2	1/2	0/2
			mal 1/2	0/1	1/1	0/1	0/1
GAS expressing	Pancreas	F 8/8	ben 2/8	2/2	1/2	2/2	2/2
			mal 6/8	4/6	3/6	2/6	3/6
	Duodenum	F 5/10	ben 2/5	2/2	2/2	1/2	0/2
			mal 3/5	3/3	3/3	3/3	1/3
		NF 5/10	ben 5/5	5/5	5/5	5/5	0/5

INS insulin, *GLU* glucagon, *SOM* somatostatin, *PP* pancreatic polypeptide, *CAL* calcitonin, *GAS* gastrin, *NET* neuroendocrine tumor, *ISL1* islet 1 gene product, *PDX1* pancreatico-duodenal homeobox 1 gene product, *NGN3* neurogenin 3 gene product, *CDX2* CDX-2 homeobox gene product

tumors are known for their preferential location in the ampullary region, an area that is intimately connected with the pancreas.

While we were able to relate some TF signatures to hormonally defined P-NET and D-NET entities, it was not possible to clearly distinguish between duodenal and pancreatic NETs. This observation was somewhat unexpected, since in our previous study on the expression of ISL1 in gastroenteropancreatic NETs, it appeared that this TF was virtually specific to P-NETs. However, already in this study we noticed that two of the four D-NETs tested were positive for ISL1 and these two tumors were gastrinomas [15]. A similar result was reported by La Rosa et al. [1]). ISL1 is therefore a marker that, in addition to P-NETs, also labels most D-NETs, particularly if they produce gastrin. Conversely, the expression of CDX2 is not restricted to D-NETs, as representatives of intestinal tumors, but may also occur in P-NETs, particularly if they belong to the gastrinoma group. These findings imply that predicting that the pancreas or the duodenum as the site of origin of a liver metastasis of a gastrin-positive NET is not possible on the basis of their positivity for ISL1 or CDX2.

A comparison of the expression of the four TFs in individual tumors belonging to the various entities reveals differences in extent and intensity in about 20% of the cases. The explanation for these differences might lie in technical criteria, since the quality of the TF staining is sensitive to factors related to tissue preservation, antigen retrieval, and antibody source. Another explanation for the inhomogeneous TF findings in some tumors within a group is tumor heterogeneity. Although the NETs in our series were mostly monohormonal, implying that they predominantly expressed only one hormone out of the seven hormones that were regularly tested, a significant expression of more than one hormone was recognized in about 15% of the cases. As multihormonality may be an indication that the tumor originated from different cell lineages, it may also explain the fact that the TF expression was never completely homogeneous in the examined tumor entities, although we were not able to correlate a multihormonal tumor with a particular TF signature. Another explanation for an "aberrant" TF expression pattern could be the plasticity of neoplastic cells enabling them to go through various phenotypical changes. These considerations are also valid for the interpretations of findings reported in other studies on TFs in P-NETs and D-NETs, which vary to some extent from those in our investigations [16, 17].

In summary, the signatures of the four TFs, ISL1, PDX1, NGN3, and CDX2, recognize hormonally defined NET entities of the pancreas and duodenum and may contribute to their identification as special D-NETs or P-NETs. The TF signatures point to an origin from embryologically determined and hormonally committed precursor cells of the various cell lineages from which the tumors may derive. The TF signatures do not allow a distinction between P-NETs and D-NETs.

Conflict of interest We declare that we have no conflict of interest.

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