Sonic hedgehog and pancreatic-duodenal homeobox 1 expression distinguish between duodenal and pancreatic gastrinomas

Volker Fendrich1, Ricarda Ramerth1, Jens Waldmann1, Katja Maschuw1, Peter Langer1, Detlef K Bartsch1, Emily P Slater1, Annette Ramaswamy2 and Matthias Rothmund1

Departments of 1Surgery and 2Pathology, Philipps-University Marburg, Baldingerstraße, D-35043 Marburg, Germany

(Correspondence should be addressed to V Fendrich; Email: fendrich@med.uni-marburg.de)

Abstract

Some 80–90% of gastrinomas are located in the gastrinoma triangle, which includes the duodenum, the pancreatic head, and the hepatoduodenal ligament. The natural history of the tumors depends on their origin. Duodenal gastrinomas are much less aggressive than pancreatic primaries and infrequently develop liver metastases. The reason therefore is unclear. The transcription factor pancreatic-duodenal homeobox 1 (Pdx1) is important in differentiation and development of the pancreas and duodenum. In embryonic development, Sonic hedgehog (Shh) expression establishes a sharp molecular boundary, which allows for the proper patterning of the duodenal and pancreatic epithelium. Pancreatic polypeptide (PP) is expressed in pancreatic islets and is known to be expressed in pancreatic endocrine tumors. This study aims to clarify the expression pattern of Pdx1, Shh, and PP in duodenal and pancreatic gastrinomas. Tissue from 15 patients with duodenal and from 11 patients with pancreatic gastrinomas that underwent surgery between 1987 and 2007 at our institution because of a gastrinoma were evaluated by immunohistochemistry (IHC). Furthermore, tissue from lymph node metastases from two patients with a so far undetected primary gastrinoma was analyzed. IHC revealed strong Pdx1 expression in pancreatic gastrinomas, but not in duodenal gastrinomas. By contrast, there was no Shh expression detectable in pancreatic gastrinomas, but found in all duodenal gastrinomas. This pattern was also true for associated metastases. Shh expression combined with absence of Pdx1 expression in lymph node metastases from patients with an unknown location of the primary suggests a so far undetected duodenal gastrinoma. We show for the first time that only pancreatic, but not duodenal gastrinomas express Pdx1. Moreover, only duodenal gastrinomas express Shh, suggesting a different genetic background of these two tumors. Whereas the expression of Pdx1 in pancreatic gastrinomas might suggest their endocrine origin from islets, duodenal gastrinomas develop from a Pdx1 negative cell cluster. The expression pattern of Pdx1, Shh, and PP in resected metastases can help to locate an otherwise undetected primary gastrinoma.

Endocrine-Related Cancer (2009) 16 613–622

Introduction

Gastrinomas, which are responsible for Zollinger–Ellison syndrome (ZES; Zollinger & Ellison 1955), were originally described as pancreatic neuroendocrine tumors, but since two decades it is known that they are most commonly located within the wall of the duodenum. Eighty to ninety percent are located in the so-called gastrinoma triangle, which includes the duodenum, the pancreatic head and the hepatoduodenal ligament (Stabile et al. 1987). Eighty percent are
situated in the first and second part of the duodenum (Hoffmann et al. 2005) correlating with the fact that this portion of the duodenum contains the majority of G cells, which are thought to be the cell of origin for most duodenal gastrinomas (Zogakis et al. 2003).

The origin of pancreatic gastrinomas has been an enigma. Attempts to detect gastrin in the normal pancreas had so far either failed or resulted in the misidentification of somatostatin cells as gastrin cells (Brand & Fuller 1988). Bardram et al. (1990) showed for the first time that human pancreatic tissue contained progastrin. Thus, gastrinomas illustrate a phenotypic characteristic of neoplasia; namely, the expression of genes which are only transiently active during fetal development. Patients with sporadic ZES are found to have a solitary duodenal or pancreatic gastrinoma. In the remaining patients, ZES is part of multiple endocrine neoplasia type 1 (MEN1) syndrome (Pipeleers-Marichal et al. 1990, Thompson 1998). Size varies with the site of the tumor; pancreatic gastrinomas are often larger than 1 cm, whereas gastrinomas of the duodenum are usually smaller (Donow et al. 1991, Sugg et al. 1993). Imaging studies fail to localize the tumor in 80% of duodenal microgastrinomas (Zogakis et al. 2003). By contrast, such studies identify 50–72% of pancreatic gastrinomas (Fendrich et al. 2007). Whereas pancreatic gastrinomas can be readily identified at exploration, duodenotomy is essential to identify duodenal gastrinomas (Sugg et al. 1993, Norton & Jensen 2004). However, the primary duodenal lesion is sometimes not identified and only nodal metastases are found. In fact, even after reoperation ZES still persists (Fendrich et al. 2006). In this case, knowledge of the potential anatomic location of the primary tumor would allow the surgeon to focus on the pancreas or the duodenum. An elegant solution to this problem would be if resected lymph node metastases expressed a marker that could distinguish between duodenal and pancreatic gastrinoma. So far, such a marker is unknown.

Duodenal tumors are not only smaller, but are also less likely to metastasize to the liver and have a better prognosis than pancreatic gastrinomas (Donow et al. 1991, Imamura et al. 1992, Yu et al. 1999, Klöppel et al. 2007). Furthermore, patients with a ZES-related death were more likely to have a gastrinoma in the pancreas and less likely to have a duodenal gastrinoma (Yu et al. 1999). The reason for this is unclear.

The gene, pancreatic-duodenal homeobox 1 (Pdx1), belongs to the ParaHox gene family of transcription factors. In mouse embryos, at E9.5, Pdx1 expression marks the dorsal and ventral pancreatic buds and the duodenal endoderm between them (Guz et al. 1995). In the adult, Pdx1 expression is maintained in the duodenal epithelium (Miller et al. 1994, Guz et al. 1995) and in the insulin-secreting islet β-cells (Offield et al. 1996). It is also found in stomach and the common bile duct, suggesting that it fulfills different roles depending on the presence of other differentiation factors (Guz et al. 1995, Offield et al. 1996, Stoffers et al. 1999). Pdx1 expression in non-islet adult pancreatic tissue has been observed in many pathologic conditions that involve reactivation of embryonic signaling pathways, such as cancer and exocrine pancreatic injury and regeneration (Song et al. 1999, Jensen et al. 2005, Liu et al. 2007, Fendrich et al. 2008).

In mouse mid-gestational embryos, Sonic hedgehog (Shh) is expressed in nearly all epithelial cells lining the alimentary canal and its function is critical for proper foregut and gastrointestinal development. By contrast, Shh is excluded from the developing pancreas, but remains expressed in the surrounding stomach and duodenal epithelium (Hebrok et al. 2000, Cano et al. 2007). Thus, Shh expression establishes a sharp molecular boundary, which allows for the proper patterning of the duodenal and pancreatic epithelium. Furthermore, overexpression of Shh within the developing pancreas of transgenic Pdx1–Shh mice leads to attenuation of pancreatic phenotype and induction of an intestinal differentiation program (Apelqvist et al. 1997). The pancreatic mesoderm of Pdx1–Shh mice is transformed into an intestinal mesenchyme, replete with a bi-layered mantle of smooth muscle as is seen in the duodenum.

The majority of pancreatic endocrine tumors express and/or secret pancreatic polypeptide (PP; Strodel et al. 1984). PP was discovered in 1968, when Kimmel et al. (1968), while purifying chicken insulin, found a new peptide hormone that they named ‘pancreatic polypeptide’. In mammals, virtually all of the PP-producing cells are located in the pancreas mainly within the islets, located in the periphery, and wedged between the A and B cells.

In the present study, we analyzed the expression pattern of Pdx1, Shh, and PP in duodenal and pancreatic gastrinomas. For the first time, we show that only pancreatic gastrinomas and their metastases are expressing Pdx1, but not Shh reflecting their pancreatic origin. By contrast, duodenal gastrinomas lack the expression of Pdx1 but expressing Shh, suggesting that duodenal and pancreatic gastrinomas are different tumor entities having nothing but the same hormone expression in common. The gene, pancreatic-duodenal homeobox 1 (Pdx1), belongs to the ParaHox gene family of transcription factors. In mouse embryos, at E9.5, Pdx1 expression marks the dorsal and ventral pancreatic buds and the duodenal endoderm between them (Guz et al. 1995). In the adult, Pdx1 expression is maintained in the duodenal epithelium (Miller et al. 1994, Guz et al. 1995) and in the insulin-secreting islet β-cells (Offield et al. 1996). It is also found in stomach and the common bile duct, suggesting that it fulfills different roles depending on the presence of other differentiation factors (Guz et al. 1995, Offield et al. 1996, Stoffers et al. 1999). Pdx1 expression in non-islet adult pancreatic tissue has been observed in many pathologic conditions that involve reactivation of embryonic signaling pathways, such as cancer and exocrine pancreatic injury and regeneration (Song et al. 1999, Jensen et al. 2005, Liu et al. 2007, Fendrich et al. 2008).

In mouse mid-gestational embryos, Sonic hedgehog (Shh) is expressed in nearly all epithelial cells lining the alimentary canal and its function is critical for proper foregut and gastrointestinal development. By contrast, Shh is excluded from the developing pancreas, but remains expressed in the surrounding stomach and duodenal epithelium (Hebrok et al. 2000, Cano et al. 2007). Thus, Shh expression establishes a sharp molecular boundary, which allows for the proper patterning of the duodenal and pancreatic epithelium. Furthermore, overexpression of Shh within the developing pancreas of transgenic Pdx1–Shh mice leads to attenuation of pancreatic phenotype and induction of an intestinal differentiation program (Apelqvist et al. 1997). The pancreatic mesoderm of Pdx1–Shh mice is transformed into an intestinal mesenchyme, replete with a bi-layered mantle of smooth muscle as is seen in the duodenum.

The majority of pancreatic endocrine tumors express and/or secret pancreatic polypeptide (PP; Strodel et al. 1984). PP was discovered in 1968, when Kimmel et al. (1968), while purifying chicken insulin, found a new peptide hormone that they named ‘pancreatic polypeptide’. In mammals, virtually all of the PP-producing cells are located in the pancreas mainly within the islets, located in the periphery, and wedged between the A and B cells.

In the present study, we analyzed the expression pattern of Pdx1, Shh, and PP in duodenal and pancreatic gastrinomas. For the first time, we show that only pancreatic gastrinomas and their metastases are expressing Pdx1, but not Shh reflecting their pancreatic origin. By contrast, duodenal gastrinomas lack the expression of Pdx1 but expressing Shh, suggesting that duodenal and pancreatic gastrinomas are different tumor entities having nothing but the same hormone expression in common.
Materials and methods

Patients

Thirty-five patients underwent surgery for duodenal or pancreatic gastrinoma and/or metastases between 1987 and April 2008 at the Department of Surgery of the Philipps-University Marburg. Seven patients had to be excluded from the study due to unavailable tissue for immunohistochemical analysis. Hence, tumor tissue from 15 patients with duodenal gastrinomas and from 11 patients with pancreatic gastrinomas was analyzed. Nineteen patients had sporadic gastrinoma, whereas nine patients had a MEN1-associated gastrinoma. MEN1 gene mutation analysis was performed by Taq cycle sequencing using an automated sequencer (ABI 310 Genetic Analyzer, Perkin Elmer, Waltham, MA, USA) as described previously by our group (Bartsch et al. 2005).

Furthermore, tissue from lymph node metastases from two patients with so far undetected primary gastrinomas was analyzed. The clinical records of all patients with at least one operation during this time range were analyzed with special regard to patient demographics, clinical characteristics, pathological findings, and long-term follow-up. Since 1997, the majority of patients were followed annually by biochemical testing, abdominal computed tomography, endoscopic ultrasonography, and somatostatin-receptor-scintigraphy at our hospital and the follow-up resulted from the most recent examination.

Operative procedures

Patients underwent operative exploration to localize and resect a primary gastrinoma and lymph node or other metastases. The abdominal cavity was systematically explored for the evidence of disease. A Kocher maneuver was performed to fully mobilize the head of the pancreas and duodenum and the lesser sac was opened to examine the pancreatic body and tail. The duodenum and pancreas were carefully palpated. Patients with no evidence for pancreatic gastrinomas underwent longitudinal duodenotomy. If a primary tumor was not on the medial duodenal wall, it was elliptically excised with a margin of 2–3 mm. Pancreatic gastrinomas were either treated by distal pancreatic resection or pylorus-preserving pancreatico-duodenectomy (PPPD) with regional lymph node dissection. For MEN1–ZES either a distal pancreatic resection to the level of the portal vein with enucleation of any tumors in the pancreatic head, a duodenotomy with excision of any tumors in the first to fourth portion of the duodenum and a peripancreatic lymph node dissection as suggested by Thompson (1998) was routinely performed until 1997. Since then, we prefer a PPPD with lymphadenectomy when the source of gastrin secretion could be regionalized to the pancreatic head region by preoperative selective arterial secretin injection angiography (Imamura et al. 1987).

Pathology

Pathologic diagnosis of a primary duodenal or pancreatic gastrinoma was made for all patients by immunohistochemical analysis for the presence of gastrin. The size of the tumor was measured and the largest diameter was documented. Lymph nodes were evaluated in a similar manner. Pathology reports were reviewed in a retrospective fashion for the size of the primary gastrinoma and the presence of positive lymph nodes.

Immunohistochemical analysis of gastrinomas

For immunolabeling, formalin-fixed and paraffin embedded archival tumor samples and corresponding normal tissues were stained as previously described (Esni et al. 2004). Briefly, slides from archived gastrinomas were heated to 60 °C for 1 h, deparaffinized using xylene, and hydrated by a graded series of ethanol washes. Antigen retrieval was accomplished by microwave heating in 10 mM sodium citrate buffer of pH 6.0 for 10 min. For immunohistochemistry (IHC), endogenous peroxidase activity was quenched by 10 min incubation in 3% H₂O₂. Non-specific binding

www.endocrinology-journals.org
was blocked with 10% serum. Sections were then probed with anti-rabbit Pdx1 (Chemicon, Temecula, CA, USA) in a dilution with 1:100 overnight at 4°C. For IHC, bound antibodies were detected using the avidin–biotin-complex (ABC) peroxidase method (ABC Elite Kit, Vector Labs, Burlingame, CA, USA). Final staining was developed with the Sigma FAST DAB peroxidase substrate kit (Sigma). Pancreatic islets from normal pancreatic tissue samples from our tissue bank were used as positive controls along with each batch of Pdx1 IHC staining.

Statistical analysis

Log-rank test was applied to identify significant differences. P values <0.05 were considered statistically significant. Data were analyzed using SPSS software (Version 11; SPSS, Inc., Chicago, IL, USA).

Results

Patients and clinical characteristics

The clinical characteristics for the identified patients are given in Table 1. A total of 28 patients with resected gastrinomas were included in the study. For the evaluation of Pdx1 expression, 12 males and 16 females with a median age of 49 years (range 28–73 years) at the time of surgery were included in this study. Eighteen patients had sporadic gastrinomas, whereas 10 patients had MEN1-gastrinomas. The patients with sporadic disease were older than patients with MEN1 syndrome (52 vs 45 years, $P \leq 0.05$) at time of surgery. Duodenal gastrinomas were significantly smaller than pancreatic gastrinomas (6 vs 24 mm, $P \leq 0.05$). Twenty-four (86%) patients had histologically verified malignant tumors as characterized by infiltrating growth and/or the presence of lymph node or distant metastases.

### Table 1 Results of pancreatic-duodenal homeobox 1 (Pdx1), Shh and PP immunohistochemistry in 28 patients with gastrinomas

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>MEN1</th>
<th>Site of gastrinaoma</th>
<th>Tumor size (mm)</th>
<th>Tissue analyzed</th>
<th>Pdx1 IHC</th>
<th>Shh IHC</th>
<th>PP IHC</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>M</td>
<td>No</td>
<td>Pancreas</td>
<td>33</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>97/AWD</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>3</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>112/NED</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>F</td>
<td>No</td>
<td>Pancreas</td>
<td>−</td>
<td>Liver-met.</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>132 NED</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>M</td>
<td>No</td>
<td>Duodenum</td>
<td>10</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>165/AWD</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>15</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>101/NED</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>8</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>238/AWD</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>No</td>
<td>Unknown</td>
<td>−</td>
<td>LN-metastasis</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>47/AWD</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>M</td>
<td>No</td>
<td>Pancreas</td>
<td>43</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>57/DOD</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>F</td>
<td>No</td>
<td>Unknown</td>
<td>−</td>
<td>LN-metastasis</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>71/AWD</td>
</tr>
<tr>
<td>10</td>
<td>64</td>
<td>F</td>
<td>No</td>
<td>Pancreas</td>
<td>40</td>
<td>Primary tumor</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>3/DOD</td>
</tr>
<tr>
<td>11</td>
<td>73</td>
<td>F</td>
<td>No</td>
<td>Pancreas</td>
<td>20</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>88/NED</td>
</tr>
<tr>
<td>12</td>
<td>59</td>
<td>M</td>
<td>No</td>
<td>Pancreas</td>
<td>10</td>
<td>Primary tumor</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>15/DURC</td>
</tr>
<tr>
<td>13</td>
<td>70</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>4</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>153/NED</td>
</tr>
<tr>
<td>14</td>
<td>43</td>
<td>M</td>
<td>No</td>
<td>Pancreas</td>
<td>−</td>
<td>Liver-met.</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>180/AWD</td>
</tr>
<tr>
<td>15</td>
<td>58</td>
<td>F</td>
<td>No</td>
<td>Pancreas</td>
<td>−</td>
<td>Liver-met.</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>1/DOD</td>
</tr>
<tr>
<td>16</td>
<td>52</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>5</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>58/NED</td>
</tr>
<tr>
<td>17</td>
<td>46</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>5</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>15/AWD</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>F</td>
<td>No</td>
<td>Duodenum</td>
<td>8</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>15/AWD</td>
</tr>
<tr>
<td>19</td>
<td>59</td>
<td>F</td>
<td>No</td>
<td>Pancreas</td>
<td>22</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>5/NED</td>
</tr>
<tr>
<td>20</td>
<td>48</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>10</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>152/AWD</td>
</tr>
<tr>
<td>21</td>
<td>49</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>3</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>117/AWD</td>
</tr>
<tr>
<td>22</td>
<td>29</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>8</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>54/AWD</td>
</tr>
<tr>
<td>23</td>
<td>46</td>
<td>F</td>
<td>Yes</td>
<td>Duodenum</td>
<td>3</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>126/AWD</td>
</tr>
<tr>
<td>24</td>
<td>48</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>6</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>56/AWD</td>
</tr>
<tr>
<td>25</td>
<td>47</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>3</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>118/AWD</td>
</tr>
<tr>
<td>26</td>
<td>45</td>
<td>F</td>
<td>Yes</td>
<td>Pancreas</td>
<td>25</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>93/AWD</td>
</tr>
<tr>
<td>27</td>
<td>32</td>
<td>F</td>
<td>Yes</td>
<td>Pancreas</td>
<td>12</td>
<td>Primary tumor</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>160/AWD</td>
</tr>
<tr>
<td>28</td>
<td>50</td>
<td>M</td>
<td>Yes</td>
<td>Duodenum</td>
<td>6</td>
<td>Primary tumor</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>21/AWD</td>
</tr>
</tbody>
</table>

MEN1, multiple endocrine neoplasia type 1; LN-metastasis, lymph node metastasis; Liver-met., liver metastasis; DOD, dead of disease; AWD, alive with disease; NED, no evidence for disease; DURC, death of unrelated cause; IHC, immunohistochemistry; Shh, sonic hedgehog; PP, pancreatic polypeptide.
Pdx1 is expressed in pancreatic gastrinomas and their associated metastases

Immunohistochemical staining revealed expression of Pdx1 in all pancreatic gastrinomas tested (Table 1 and Fig. 1C). Pdx1 positive tumors cells showed a typical nuclear staining pattern as seen in normal islet cells and were found to be distributed throughout large areas of the tumors. Pdx1 was also expressed in all associated lymph node (n = 2) or liver metastases (n = 3) from pancreatic gastrinomas analyzed (Table 1).

Shh is not expressed in pancreatic gastrinomas and their associated metastases

In all pancreatic gastrinomas analyzed, expression of Shh was not detected (Table 1 and Fig. 1D). Furthermore, all the associated metastases did not show any expression of Shh.

PP is expressed in some pancreatic gastrinomas

PP expression was found in 7 out of 11 pancreatic gastrinomas (Table 1 and Fig. 1E). Furthermore, we found a diffuse PP hyperplasia in a ZES–MEN1 patient with a pancreatic gastrinoma (Fig. 4A and B).

Pdx1 is not expressed in duodenal gastrinomas and their associated metastases

In all duodenal gastrinomas analyzed, Pdx1 expression was absent (Table 1 and Fig. 2C). Pdx1 was also undetectable in all associated lymph node metastases (n = 3) from duodenal gastrinomas analyzed (Table 1, data not shown).

Shh is expressed in duodenal gastrinomas and their associated metastases

In contrast to pancreatic gastrinomas, Shh was expressed in all duodenal gastrinomas (Table 1 and

Figure 1 IHC staining of pancreatic gastrinoma. (A) H&E staining (10×). (B) H&E staining (40×). (C) Representative example of Pdx1 expression in a pancreatic gastrinoma, showing a typical nuclear staining pattern for the transcription factor. (D) Absence of Sonic hedgehog staining in the same tumor. (E) Positive staining for pancreatic polypeptide in some pancreatic gastrinoma cells. Full colour version of this figure available via http://dx.doi.org/10.1677/ERC-08-0204.
Fig. 2 D). Tumor cells expressing SHH were found to be distributed throughout large areas of the tumors. Shh was also detectable in all associated lymph node metastases \((n=3)\). As expected, SHH expression was also seen in normal intestinal epithelium (Fig. 2D).

**PP is not expressed in duodenal gastrinomas**

Expression of PP was not seen in any duodenal gastrinoma examined (Table 1 and Fig. 3E).

**Expression of Pdx1, Shh, and PP in metastases from gastrinomas with unknown origin**

Pdx1 expression was absent in both lymph node metastases from the two patients with an unknown location of the primary (Table 1 and Fig. 3B). Furthermore, we found expression of Shh in both cases suggesting a so far undetected duodenal gastrinoma (Table 1 and Fig. 3C). PP was not expressed on both metastases (Fig. 3D).

**Discussion**

After proving the existence of gastrin expression in adult pancreatic tissue, it became clear that pancreatic gastrinomas do not develop by ectopic dedifferentiation of transcription mechanisms, but rather by acceleration of already existing translational and post-translational processing mechanisms (Bardram et al. 1990). Sporadic duodenal gastrinomas usually arise from the first part of the duodenum and are located in the submucosa (Guz et al. 1995). Although studies have shown that both
locations are equally malignant (40–70% metastases), and the post-operative disease-free rate is similar (Weber et al. 1995, Norton et al. 1999), the biological behavior of pancreatic and duodenal gastrinomas is quite different. Pancreatic gastrinomas usually have a diameter of 2 cm or more. Metastasis of pancreatic gastrinomas to regional lymph nodes is found in ~60% of patients at the time of diagnosis and liver metastases occur more frequently (10–20%) than in duodenal gastrinomas (Stabile & Passaro 1985, Norton et al. 2006). Thus, the 10-year survival rate is worse in patients with pancreatic gastrinomas (57%) than in patients with duodenal gastrinomas (84%; Weber et al. 1995, Yu et al. 1999, Norton & Jensen 2004). Despite the fact that most of the duodenal gastrinomas are smaller than 1 cm, metastases to regional lymph nodes are already found in 60–80% of the patients at the time of diagnosis. It seems that periduodenal lymph node metastases may grow faster than their duodenal primary tumors and thus may form large tumors that are easily recognized, in contrast to the duodenal primary tumors (Anlauf et al. 2006). Unfortunately, the molecular pathogenesis of gastrinomas contributing to these differences is largely unknown (Fendrich et al. 2007). Molecular data on gastrinoma have been accumulating in recent years, but the genetic basis of endocrine tumor development and progression is still poorly understood (Chen et al. 2003, 2004, Furukawa et al. 2005).

Our study is the first to show that Pdx1 is expressed only in pancreatic, but not in duodenal gastrinomas (Figs 1C and 2C).

The Pdx1 gene was first described by Miller et al. (1994) and codes for one of the earliest transcriptional factors detected within the developing pancreatic epithelium (Offield et al. 1996). At E9.5, Pdx1 positive cells are seen in the ventral pancreatic primordia and at E11.5, groups of cells adjacent to the pancreatic duct also immunostain for Pdx1. At E17.5, Pdx1 positive cells in the pancreas were seen exclusively within islets (Peshavaria et al. 1994). In adult tissue, Pdx1 is mainly found in PP and insulin-expressing β-cells (Leonard et al. 1993, Miller et al. 1994).

In the duodenum of older embryos and adults, almost all cells forming the simple columnar epithelium that line the villi were Pdx1 positive, whereas the crypt cells did not contain the homeo-protein. Cells of the other layers of the mucosa as well as cells of the submucosa, muscularis and adventitial layers of the wall of the duodenum also lacked Pdx1 expression (Miller et al. 1994, Guz et al. 1995).

The observations of our study suggest that Pdx1 expression might distinguish between duodenal and pancreatic gastrinomas. Driven by these results, we
searched for another possibility to differentiate these two tumors by IHC. The next marker we analyzed was Shh. The fundamental roles of Hh signaling proteins in embryonic patterning have been established in multicellular organisms ranging from insects to man. The \textit{Hh} gene initially was identified as required for segmental patterning in the \textit{Drosophila} embryo (Nusslein-Volhard & Wieschaus 1980), and three mammalian orthologs – Sonic, Indian, and Desert hedgehog – have been subsequently identified that establish morphologic gradients essential for axial patterning of the mammalian embryo. Interestingly, during gastrointestinal development, Shh expression is found within the duodenal tissue that connects the opposing dorsal and ventral buds of the pancreas, resulting in a sharp molecular boundary that separates the duodenal/stomach epithelium from pancreatic tissue (Apelqvist \textit{et al.} 1997, Hebrok \textit{et al.} 2000). This pattern, expression in stomach and duodenum and exclusion in pancreatic tissue, is maintained throughout organogenesis (Hebrok \textit{et al.} 2000, Ramalho-Santos \textit{et al.} 2000). Now, we found that this expression pattern remains true for pancreatic and duodenal gastrinomas. Whereas Shh expression was absent in all pancreatic gastrinomas, all duodenal gastrinomas showed a strong expression for Shh (Figs 1D and 2D).

In addition, we stained all gastrinomas and metastases for PP. As reported by others (Larsson \textit{et al.} 1976, Strodel \textit{et al.} 1984), PP is expressed in pancreatic endocrine tumors. In line with these results, we found expression of PP in a part of pancreatic gastrinomas (Fig. 1E). Interestingly, we found a diffuse PP hyperplasia in a MEN1 patient as described in the literature (Fig. 4). By contrast, we did not find any PP expression in duodenal gastrinomas.

Two patients from our study population have so far undetected primary gastrinomas. Both underwent an abdominal exploration because of proven ZES, and in both cases lymph node metastases had been resected. Even experienced surgeons are sometimes not able to identify a primary tumor in ZES patients although they had lymph nodes containing gastrinoma tissue (Anlauf \textit{et al.} 2005, 2006, Fendrich \textit{et al.} 2007). Because in some of these patients, symptomatic and/or biochemical cure seemed to occur after resection of lymph nodes involved by gastrinoma, the existence of primary lymph node gastrinomas was suggested (Norton \textit{et al.} 2003). This view, however, was challenged when it was demonstrated that duodenal gastrinomas were commonly very small and easily overlooked. Furthermore, we and others have shown that even small duodenal gastrinomas could give rise to extensive lymph node metastases (Pipeleers-Marichal \textit{et al.} 1990, Akerstrom \textit{et al.} 2002, Bartsch \textit{et al.} 2005). In the lymph node metastases investigated in this study by IHC, Pdx1 was not expressed (Fig. 3B). By contrast, both metastases revealed a strong expression of Shh (Fig. 3C), suggesting a so far undetected duodenal gastrinoma.

In conclusion, we show for the first time that only pancreatic, but not duodenal gastrinomas express Pdx1. Moreover, only duodenal gastrinomas express Shh, suggesting a different genetic background of these two tumors. Whereas the expression of Pdx1 in pancreatic gastrinomas might suggest their endocrine origin from islets, duodenal gastrinomas develop from a Pdx1 negative cell cluster. This might be the reason for their different biological behavior. Furthermore, the expression pattern of Pdx1, Shh, and PP in resected metastases can help to locate an otherwise undetected primary gastrinoma.
Declaration of interest
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Funding
V F was supported by a Research Grant from the University Medical Center Giessen and Marburg.

References
V Fendrich et al.: Sonic hedgehog and Pdx1 in gastrinomas


