Ampullary Somatostatinoma: Psammomatous Variant of Gastrointestinal Carcinoid Tumor—An Immunohistochemical and Ultrastructural Study. Report of a Case and Review of the Literature

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A 28-year-old man presented with epigastric pain and obstructive jaundice associated with a histologically and immunologically unusual variant of carcinoid tumor involving the ampulla of Vater. The tumor contained abundant psammoma bodies and exhibited immunoreactivity only for somatostatin. Immunoperoxidase studies for insulin, glucagon, vasoactive intestinal peptide, calcitonin, serotonin, and ACTH had negative results. In contrast to most somatostatinomas of pancreatic origin, clinically this ampullary somatostatinoma was not accompanied by features of the somatostatinoma syndrome.

A literature review of the clinical and hormonal features in reported cases of gastrointestinal and pancreatic somatostatinomas is presented. (Key words: Somatostatin; Carcinoid; Immunoperoxidase; Ampulla of vater) Am J Clin Pathol 1983; 80: 755–761

IN 1973, somatostatin first was isolated and characterized physiologically as a hypothalamic hormone with an inhibitory effect on the release of growth hormone. Subsequent investigations have demonstrated the presence of somatostatin-containing cells throughout the central and peripheral nervous system, the gastrointestinal tract, and pancreas. In all these sites, somatostatin has been found to function as an inhibitory hormone.

Recent immunohistochemical studies have documented the presence of somatostatin-containing cells in tumors of pancreatic, pulmonary, adrenal, and thymic origin. Somatostatin generally has represented one of a number of hormones identified in these tumors. A small number of neoplasms also have been described in which somatostatin-containing cells predominate ("somatostatinomas"). Most tumors have involved the pancreas (Table 1) and were associated with a clinical syndrome of dyspepsia, steatorrhea, mild diabetes mellitus, and cholelithiasis. The clinical symptoms are at least partially attributable to somatostatin's inhibitory action on the secretion of gastrin, secretin, insulin, and cholecystokinin.

Somatostatin-producing tumors involving the gastrointestinal tract are more unusual than those of pancreatic origin. The few described have occurred in the duodenum or rarely in the jejunum.

This case report describes the first documented somatostatin-producing neoplasm of the ampulla of Vater. This carcinoid type tumor was associated with a clinical presentation of obstructive jaundice and was histologically highly unusual due to the presence of abundant psammoma bodies.

Materials and Methods

Tissues for routine histology and for immunohistochemical studies were fixed in 10% neutral buffered formalin. Fontana Mason stain was used to detect argentaffin cells. The DeGrandi modification of the Grimelius stain was employed to identify argyrophilic cells. The latter stain was modified further by one of us (GSP), increasing the silver nitrate solution to 0.5 g% and the incubation time by one hour (total four hours), in order to demonstrate somatostatin-containing cells.

Immunoperoxidase studies of paraffin sections were performed using a triple bridge indirect technic, as previously described. Briefly, deparaffinized sections were incubated for 30 minutes in an 0.5% solution of hydrogen peroxide in methanol to eliminate endogenous peroxidase activity. Sections then were washed and placed in 0.1 M Tris buffer, pH 7.6. Sequential 30-minute incubations then were performed with 1) specific antibody, 2) swine anti-rabbit IgG antiserum* and 3) horseradish peroxidase–rabbit antihorseradish peroxidase immune complexes.

* Dakopatts: A/S Copenhagen, Denmark; U.S. distributor, Accurate Chemical and Scientific Co., Westbury, New York.
### Table 1. Clinical Features in 11 Pancreatic and Six Gastrointestinal Somatostatinomas

<table>
<thead>
<tr>
<th>Reported Cases (Reference Number)</th>
<th>Age</th>
<th>Sex</th>
<th>Location of Tumor</th>
<th>Liver and/or Lymph Node Metastasis</th>
<th>Glucose Intolerance and/or Diabetes</th>
<th>Cholelithiasis</th>
<th>Diarrhea and/or Steatorrhea</th>
<th>Additional Hormone Production*</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsson and associates(^{12})</td>
<td>55</td>
<td>F</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Negative for c, d, e, f, g, i, k, l, n, and p</td>
<td>Whipple’s resection</td>
<td>Died postoperatively</td>
</tr>
<tr>
<td>Ganda and co-workers(^{1,14})</td>
<td>46</td>
<td>F</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Trace of e, f, g, k, and p by tissue RIA</td>
<td>Whipple’s resection</td>
<td>NED 4 years postoperatively</td>
</tr>
<tr>
<td>Kovacs and colleagues(^{20})</td>
<td>54</td>
<td>M</td>
<td>Tail of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ACTH</td>
<td>Surgery</td>
<td>Died postoperatively</td>
</tr>
<tr>
<td>DeNutte and associates(^{10})</td>
<td>56</td>
<td>F</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Negative for f, g, k, l, and p</td>
<td>Streptozotocin</td>
<td>Alive 30 weeks with tumor shrinkage</td>
</tr>
<tr>
<td>Pipeleers and co-workers(^{28})</td>
<td>70</td>
<td>F</td>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Calcitonin; negative for e, g, f, and k</td>
<td>Whipple’s resection</td>
<td>Died 4 weeks postoperatively</td>
</tr>
<tr>
<td>Galmiche and colleagues(^{11,12})</td>
<td>52</td>
<td>M</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Calcitonin; negative for a, e, f, g, k, l, and o</td>
<td>Whipple’s resection, doxorubicin, and 5-FU</td>
<td>NED 2 years postoperatively</td>
</tr>
<tr>
<td>Conlon and associates(^{6})</td>
<td>56</td>
<td>F</td>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Negative for e, f, g and p</td>
<td>Surgery, 5-FU, doxorubicin</td>
<td>Not known</td>
</tr>
<tr>
<td>Krejs and co-workers(^{21})</td>
<td>52</td>
<td>M</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Insulin; negative for d, e, f, and k</td>
<td>5-FU</td>
<td>Alive 1 year postoperatively</td>
</tr>
<tr>
<td>Berger and co-workers(^{1})</td>
<td>67</td>
<td>F</td>
<td>Pancreas</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>+</td>
<td>Negative for e, f, g and p</td>
<td>Surgery</td>
<td>Died 5 months after diagnosis</td>
</tr>
<tr>
<td>Penman and colleagues(^{26})</td>
<td>36</td>
<td>M</td>
<td>Tail of pancreas</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Positive for ACTH and calcitonin by plasma RIA</td>
<td>Surgery</td>
<td>NED 2 years postoperatively</td>
</tr>
<tr>
<td>Penman and associates(^{26})</td>
<td>33</td>
<td>F</td>
<td>Pancreas</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Insulin; negative for d, e, f, and k</td>
<td>5-FU</td>
<td>Alive 1 year postoperatively</td>
</tr>
<tr>
<td>Wright and co-workers(^{26})</td>
<td>54</td>
<td>F</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Negative for f and g</td>
<td>5-FU, streptozocin, doxorubicin, decarbazine</td>
<td>Alive 5 months postoperatively</td>
</tr>
<tr>
<td>Axelrod and colleagues(^{2})</td>
<td>54</td>
<td>F</td>
<td>Head of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Negative for e and p by plasma RIA</td>
<td>Distal pancreatectomy</td>
<td>NED 1 year, 10 months postoperatively</td>
</tr>
<tr>
<td>Gerlock and associates(^{15})</td>
<td>35</td>
<td>F</td>
<td>Tail of pancreas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Negative for e, f, g, h, i, j, l, n, and p</td>
<td>Surgery</td>
<td>Not known</td>
</tr>
<tr>
<td>Kaneko and co-workers(^{17})</td>
<td>26</td>
<td>M</td>
<td>Duodenum</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Negative for e, f, g, h, i, j, l, n, and p</td>
<td>Distal pancreatectomy</td>
<td>NED 1½ years postoperatively</td>
</tr>
<tr>
<td>Alumets and colleagues(^{12})</td>
<td>55</td>
<td>F</td>
<td>Jejunum, 15 cm from ligament of Treitz</td>
<td>+</td>
<td>NR</td>
<td>+</td>
<td>NR</td>
<td>Gastrin</td>
<td>Surgery</td>
<td>NED 4½ years postoperatively</td>
</tr>
<tr>
<td>Dayal and associates(^{14})</td>
<td>41</td>
<td>M</td>
<td>Duodenum</td>
<td>+</td>
<td>+ (longstanding)</td>
<td>NR</td>
<td>+</td>
<td>Negative for a, b, e, f, g, k, m (all cases)</td>
<td>Whipple’s resection (all cases)</td>
<td>Died postoperatively</td>
</tr>
<tr>
<td>Current case</td>
<td>57</td>
<td>M</td>
<td>Duodenum</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>NR</td>
<td>Negative for a, b, e, f, g, k, m (all cases)</td>
<td>Surgery</td>
<td>Died postoperatively</td>
</tr>
<tr>
<td>Current case</td>
<td>49</td>
<td>M</td>
<td>Duodenum</td>
<td>+</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Negative for a, b, e, f, g, k, m, and p</td>
<td>Surgery</td>
<td>Died postoperatively</td>
</tr>
<tr>
<td>Current case</td>
<td>28</td>
<td>M</td>
<td>Ampulla of Vater</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Negative for a, b, e, f, g, k, m, and p</td>
<td>Surgery</td>
<td>Died postoperatively</td>
</tr>
</tbody>
</table>

NR = not recorded; NED = no evidence of disease.

* Immunocytochemical studies (immunoperoxidase and/or immunofluorescence unless otherwise specified). Hormones: (a) ACTH; (b) Calcitonin; (c) cholecystokinin; (d) gastric inhibitory peptide; (e) gastrin; (f) glucagon; (g) insulin; (h) met-enkephalin; (i) motilin; (j) neurotensin; (k) pancreatic polypeptide; (l) secretin; (m) serotonin; (n) substance P; (o) TRH; (p) vasoactive intestinal polypeptide.

† Also personal communication.
(PAP; Dakopatts). For studies with antiinsulin and antigu­
glucagon antisera, incubation with primary antibody was
performed overnight. Antibody localization was ef­
acted using a solution containing hydrogen peroxide and
3,3'-diaminobenzidine in Tris buffer, yielding a brown
reaction product. Sections were counterstained with
methyl green or hematoxylin, dehydrated, and mounted
with Permount®.

Specific antibodies were obtained from the following
sources: rabbit antiserum against synthetic somatostatin
from Dr. S. Reichlin, Tufts University School of Medicine,
Boston; guinea pig antiovine insulin serum from Dr. P.
Wright, University of Indiana School of Medicine, In­
dianapolis, Indiana; rabbit antiovine-antiporcine glu­
cagon serum from Dr. R. H. Unger, University of Texas
Southwestern Medical School, Dallas, Texas; rabbit anti­
calcitonin serum from Dakopatts; rabbit antiserotonin
serum from Immunonuclear Corp., Stillwater, Minnesota;
and rabbit anti-ACTH and acti-vasoactive intestinal
polypeptide sera from Immulok, Carpinteria, California.
Appropriate positive control slides were processed in all
studies. Specificity of the antisomatostatin, antiinsulin,
and antigu­glucagon antisera for distinct cell populations of
pancreatic islets has been verified previously.25 Specificity
of staining with antisomatostatin serum was verified fur­
ther in studies using antiserum that had been adsorbed
with synthetic somatostatin. Normal rabbit serum was
substituted for the specific antiserum as a negative control.

Tissue for electron microscopy was minced into 1-mm
cubes and fixed in 3% glutaraldehyde fixative in cacodylate
buffer pH 7.4. Material for conventional electron mi­
croscopy was postfixed in osmium tetraoxide, dehydrated
in alcohol, and embedded in Epon. One-micron sections
were cut and stained with toluidine blue to select areas for
thin sectioning. Thin (silver-gold) sections were cut,
stained with uranyl acetate followed by lead citrate, and
examined with a JEOL-EMS-100 electron microscope.

For immunoelectron microscopy, the osmium fixation
was eliminated. After glutaraldehyde fixation, the tissue
was dehydrated in alcohols and embedded in Epon. Thick
sections were cut and stained with toluidine blue to select
appropriate areas of thin sectioning. The thin sections
were picked up on nickel grids, then etched in 10%
aqueous hydrogen peroxide for ten minutes, and rinsed
three times with 0.1 M Tris buffer pH 7.4. Sections then
were incubated with the following antiserum for 2 hours
at room temperature: antisomatostatin, antinsi­
insulin, anti-
VIP, and antigu­glucagon, and with normal rabbit serum as
a control. Following incubation, a triple-bridge immu­
noeroxidase technic was performed similar to that used
for paraffin sections. The sections then were examined
and photographed unstained.

Clinical History
A 28-year-old man presented in June 1981 with a three- to four-week
history of increasing epigastric distress characterized by distention and
vague aches and pains, especially related to ingestion of meals. Appetite
was decreased and associated with a 10-pound weight loss. Dark urine
was noted along with light-colored stools, and jaundice became evident.
His past medical history was unremarkable. He was admitted to another
hospital where physical examination revealed only mild jaundice. Per­
tinent laboratory chem­istries included bilirubin 5 mg/dL (normal 0.1­
1.2), SGPT 95 IU/L (normal 3-28), alkaline phosphatase 450 IU/L
(normal 24-71), alpha feto-protein 20 ng/mL (normal less than 30),
and CEA 5 ng/mL (normal less than 2.5). A liver scan was normal, but
an upper gastrointestinal series showed a defect in the area of the ampulla
of Vater suggesting a periampullary mass lesion. An abdominal CT scan
revealed dilated biliary ducts consistent with extrahepatic bile duct ob­
struction. No definite mass lesions were noted in the region of the
pancreas. An exploratory laparotomy was performed, which revealed a
tumor in the ampulla of Vater as the cause of the obstructive jaundice.
A modified Whipple's procedure was performed.

The patient's postoperative course was complicated by an acute upper
gastrointestinal tract hemorrhage, which responded to clinical manage­
ment.

Subsequent follow-up including physical examination, laboratory
studies, x-rays, and abdominal CT scan have been unremarkable, except
for several cafe au lait spots that involve the right arm, abdomen, and
chest.

Pathologic Findings
Gross examination of the surgical specimen revealed a 4 X 3 X 1.5
cm polypoid oval mass, which completely occupied the ampullary region.
The overlying duodenal mucosa had a granular gray-tan appearance.
Sections through the mass demonstrated a yellow-tan moderately well­
circumscribed nodule (3.5 X 2.5 X 2.0 cm). The common bile duct and
pancreatic ducts penetrated the middle of the mass. At this point their
lumina were collapsed. The common bile duct was dilated proximally
to a circumference of 3.0 cm at the margin of resection. No mucosal
lesions were noted in either the common bile duct nor the pancreatic
duct. The pancreatic parenchyma was grossly unremarkable. Multiple
enlarged peripancreatic lymph nodes were identified.

Microscopically, the ampullary tumor was composed of irregular rib­
bons or trabeculae of uniform monomorphic polygonal or polyhedral
cells separated by thin fibrovascular septa (Fig. 1). The tumor cells
contained round-to-ovoid nuclei with fine, stippled chromatin and one
or two small nucleoli. Minimal nuclear pleomorphism was noted. The
cells had abundant granular eosinophilic cytoplasm. Intermixed with
these cords of tumor cells were ductlike spaces lined by uniform tall
columnar cells having round-to-ovoid basally oriented nuclei and areas
with a glandular growth pattern. There appeared to be a transition from
one histologic pattern into the other. Tumor extended through the mus­
cularis mucosa into the mucosa but did not extend to the mucosal
surface. Mitoses were not detected. Abundant concentrically laminated
calified psammoma bodies were present throughout the tumor (Figs.
1B, C) and in its metastases. Six of the eight peripancreatic lymph nodes
contained metastatic tumor. The pancreas, the common bile duct, and
pancreatic duct were not involved by tumor.

Histochemically, the tumor cell granules were Fontana-Masson neg­
avive (argentaffin stain) and exhibited little to no argyrophilia, employing
the DeGrandi modification of the Grimelius technic. The latter technic,
Somatostatinoma of ampulla of Vater. Carcinoid-type tumor exhibits a trabecular pattern, with overlying duodenal mucosa and uninvolved distal small ampullary ducts. Higher magnification of trabecular and ductlike patterns, with calcified psammoma bodies (arrows). Higher power of a concentrically laminated psammoma body. Hematoxylin and eosin, A (X70); B (X175); C (X700).

Fig. 2 (upper, right). The tumor cells are strongly argentophilic. A psammoma body is noted. Modified Grimelius stain (X475).

Fig. 3 (lower, right). Immunoperoxidase studies demonstrate strong immunoreactivity for somatostatin in tumor cells. Ductal epithelial cells are negative (upper right corner of illustration). Methyl green counterstain (X520).

However, did stain argentaffin cells in the duodenal mucosa. Using a further modification of this argyrophil stain (as indicated in methods section), strong staining of cytoplasmic granules was observed (Fig. 2). Immunoperoxidase studies of paraffin sections using a triple-bridge indirect technic14 revealed strong immunoreactivity of tumor cells for somatostatin (Fig. 3), and a lack of staining for insulin, glucagon, calcitonin, gastrin, vasoactive intestinal peptide, ACTH, and serotonin. The specificity of staining for somatostatin was confirmed by an absence of staining in studies employing antisomatostatin serum, which had been adsorbed with somatostatin.

Conventional transmission electron microscopy revealed features typical of an endocrine type neoplasm (Fig. 4A). Polygonal cells were filled with many round electron-dense cytoplasmic granules, 350–550 nm in diameter, with closely apposed limiting membranes (Fig. 4B) typical of those observed in type D endocrine cells (Lausanne classification; Solcia and associates29). Also present was rough endoplasmic reticulum, scattered...
mitochondria, and a small Golgi area. No filamentous structures were noted. Plasma membranes were joined by rare zona adherens. Clusters of cells were surrounded by basal lamina.

Immunoelectron microscopy with antisomatostatin serum revealed localization of the coarsely granular, electron-dense diaminobenzidine precipitate to the granules (Fig. 5A). Neither the control normal rabbit serum (Fig. 5B) nor the antisera directed against other hormones produced precipitate visible ultrastructurally.

Discussion

This report describes an endocrine tumor of the carcinoid type, immunohistochemically a somatostatinoma, which arose at the ampulla of Vater and presented clinically with obstructive jaundice. In contrast to pancreatic somatostatinomas, many of which have been associated with the somatostatinoma syndrome, i.e., dyspepsia, steatorrhea, glucose intolerance, and cholelithiasis, our patient and the few others reported with gastrointestinal somatostatinomas, failed to exhibit these signs and symptoms (Table I). The gastrointestinal tumors appeared to be hormonally inactive, with epigastric or abdominal pain as the main clinical feature. Lymph node and/or hepatic metastases were characteristic of both groups. An interesting clinical finding in our patient was the presence of cafe au lait skin lesions, unassociated with evidence of von Recklinghausen's disease. Skin lesions of this type, however, have been described with endocrine tumors, particularly those involving the ampullary region.

Most patients with somatostatinomas have been treated by surgery (Whipple's resection). Chemotherapy has been used postoperatively in many patients (streptozotocin, 5-fluorouracil, and/or doxorubicin). The outcome of these patients has varied considerably, though several patients were reportedly alive with no evidence of disease up to four years after diagnosis, even though lymph node metastases were observed. However, the small number of reported cases precludes valid assessment of therapy and ultimate prognosis.

Histologically, our ampullary tumor and several other duodenal somatostatinomas appear to represent a histologically distinct variant of carcinoid tumor with abundant psammoma bodies. By contrast, the pancreatic "somatostatinomas" exhibit features characteristic of islet cell tumors. Dayal and co-workers recently suggested that the histologically unusual psammomatous variant of gastrointestinal carcinoid is characteristic of duodenal somatostatinomas.

![Fig. 4. A (upper). Somatostatinoma. Electromicrograph reveals polygonal cells containing numerous granules, encased by a basal lamina. A capillary (top, left) is in close juxtaposition to the tumor cells. B (lower). Higher magnification of the tumor cells further defines the round electron dense cytoplasmic granules (150-500 nm), which are surrounded by a closely apposed limiting membrane Uranyl acetate and lead citrate; A (×2000); B (×8000).]
matostatinoma. Murayama and colleagues also described a psammomatus duodenal carcinoid tumor involving the ampulla of Vater, which occurred in a 27-year-old man who presented with obstructive jaundice. Although the tumor cells in their case were not evaluated for specific hormone production, electron microscopy revealed type D endocrine cells, essentially diagnostic of somatostatin production. The psammomatus type of carcinoid tumor, if unrecognized, may cause diagnostic difficulties. The presence of a glandular growth pattern in this neoplasm may suggest an adenocarcinoma. This finding, in conjunction with an absence of argyrophilia by standard technics, may add further confusion. In our case, a modified Grimelius stain was employed to demonstrate argyrophilia. Other studies have utilized the Hellerstrom-Hellman technic to define argyrophilic granules in somatostatin-containing cells.

Studies of hormonally silent intestinal and nonintestinal carcinoid tumors have shown immunoreactivity for one or more hormones. Somatostatin-containing cells have been identified in most patients, especially those of foregut derivation, suggesting that this hormone represents a useful marker for carcinoid tumors. However, somatostatin generally was not the exclusive hormone identified in most tumors. In reported cases of pancreatic somatostatinomas, other hormones also were detected in some tumors (Table 1). The three psammomatus duodenal carcinoids reported by Dayal and associates and that described in this report were immunohistochemically distinct in that immunoreactivity was observed only for somatostatin.

In summary, our report documents an unusual psammomatus variant of carcinoid tumor, which involves the ampulla of Vater, that is associated with endocrine cells exclusively involved with somatostatin production and is not accompanied by clinical features of the somatostatinoma syndrome.

Acknowledgments. The authors gratefully acknowledge the expert technical assistance of Mrs. Maureen Perry, Mrs. Despina Samiotes, and Mrs. Christine Ridolfi and the skilled secretarial assistance of Ms. Ann Benoit.

References
inhibits the secretion of immunoreactive pituitary growth hormone. Science 1973; 179:77-79


