

Case Report

Serotonin- and Somatostatin-Positive Goblet Cell Carcinoid of the Duodenum

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In the duodenum, mixed exocrine-endocrine tumors exhibiting both neuroendocrine and glandular differentiations [cf. appendiceal goblet cell carcinoids (GCCs)] are rare. We present a Japanese case with a duodenal GCC that was found during pathologic examination of a gastrectomy specimen removed for gastric mucosal cancer. The tumor was widely distributed within both the first portion of the duodenum and the gastric antrum, although mucosal involvement was observed only in the duodenum. The tumor cells formed solid nests, trabeculae, or tubules, and some displayed a goblet cell appearance. They were immunoreactive against antibodies for both serotonin and somatostatin, and showed an argentaffin reaction (similar to a "midgut" enterochromaffin cell carcinoid). Ultrastructurally, the tumor cells had an amphicrine nature. Physicians encounter GCC in the duodenum only rarely, and its discovery may be incidental. Its diagnosis will be challenging and will require careful clinical and pathologic examinations.

Key words: amphicrine tumor, duodenum, goblet cell carcinoid, serotonin, somatostatin

Due to advances in diagnostic equipment, the incidence of duodenal endocrine tumors has been found in recent years to amount to 22% of all gastrointestinal endocrine neoplasms [1]. Among such duodenal tumors, which are located mostly within the first or second part of the duodenum, gastrin cell tumors predominate, followed by somatostatin cell tumors. Although mixed exocrine-endocrine tumors showing both neuroendocrine and glandular differentiations [cf. appendiceal goblet cell carcinoids (GCCs)] exist, they are rare in this region [1]. Here, we present a Japanese case with a duodenal GCC that was found during pathologic examination of a gastrectomy

specimen removed for gastric mucosal cancer. We describe the tumor features and histogenesis, and discuss the prognosis. Our experience in this case leads us to emphasize that a diagnosis of GCC will require careful clinical and pathologic examinations. Since its discovery is likely to be incidental, its diagnosis will be challenging.

Case Report

A 61-year-old Japanese man was admitted as an emergency to the Japan Self-Defense Forces Hospital Yokosuka (JSDFHY; Yokosuka, Japan) because of repeated hematemesis. His history was unremarkable, although both of his parents had had gastrointestinal cancer. Three months before admission, he had experienced his first hematemesis for which he then

received remedies at another hospital. Hematemesis then recurred 10 days before his admission to our hospital, accompanied by difficulty in eating. On admission, his blood hemoglobin was 10.2mg/dL, and an emergency upper endoscopy revealed multiple ulcerative and erosive lesions in both the stomach and the duodenum. Of these, the open gastric ulcers were considered mainly responsible for his hematemesis. A rapid urease test and histology for *Helicobacter pylori* were both negative. Our initial diagnosis was multiple ulcers. Although both his symptoms and his open ulcers were almost resolved by administration of a proton-pump inhibitor, a shallow-depressed lesion remained in the lower gastric body (Fig. 1A). On the

other hand, no apparent findings suggesting a tumor were observed in the duodenum (Fig. 1B). Biopsy examination of tissue taken from the gastric lesion during follow-up endoscopy at the outpatient clinic revealed a poorly differentiated adenocarcinoma with signet-ring cell features. Imaging studies revealed no distant metastases, and a distal gastrectomy was performed, with the preoperative diagnosis of a gastric cancer coincident with ulcer scars.

In the distal gastrectomy specimen, in addition to 3 ulcer scars, an 18 × 11 mm mucosal cancer (poorly differentiated adenocarcinoma, without neuroendocrine differentiation) was found in the lower gastric body at a location corresponding to the shallow-

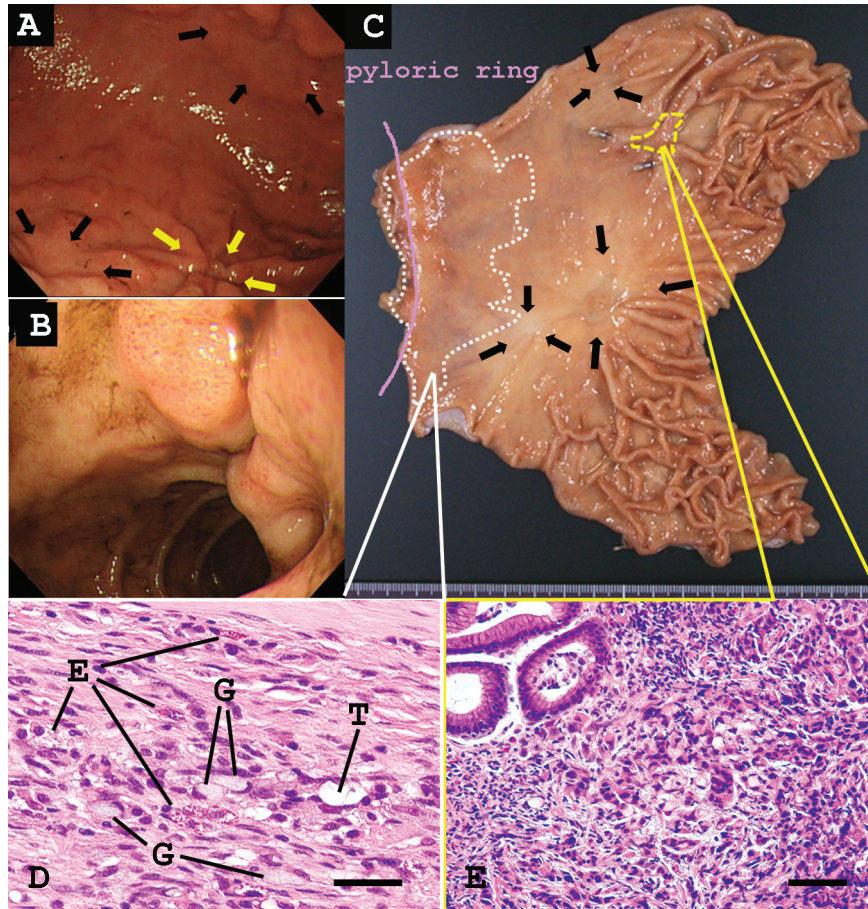


Fig. 1 A, B, Upper endoscopy revealed both a shallow-depressed lesion (yellow arrows) and multiple ulcers (black arrows) in the stomach (A) and rugal deformity in the duodenum (B). C–E, Gastrectomy specimen (C) displayed three ulcer scars with fold convergences (black arrows) and a shallow-depressed lesion (broken yellow line in C), which proved, by histology, to be an intramucosal poorly differentiated adenocarcinoma (E; hematoxylin-eosin). Broken white line in C delineates the region of the found infiltrating tumor, the perineural invasion of which into the gastric antral wall exhibited goblet cell (G), eosinophilic granular (E), and aborted tubular (T) features (D; hematoxylin-eosin). Scale bars in D and E indicate 50 μm and 100 μm, respectively.

depressed lesion (Fig. 1C, E). No tumor deposits were evident within the dissected perigastric or periduodenal lymph nodes. However, an unexpected tumor was found in the distal area (the duodenum and gastric antrum) away from the conventional gastric cancer and ulcer scars. The latter tumor exhibited both neuroendocrine and glandular differentiations, with solid nests exposed in the intramural region within the duodenal stump, and infiltrated widely into the gastric antrum (predominantly into Auerbach's nerve plexuses, without mucosal involvement) (Fig. 1C, D).

Although an examination of biopsy material obtained from the Billroth-I-anastomized duodenum at the subsequent endoscopy failed to find a residual tumor, a duodenectomy and remnant gastrectomy with a

Billroth-II anastomosis was performed as the second operation 5 weeks after the distal gastrectomy. At that time, serum tumor markers [carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 72-4, CA19-9, neuron-specific enolase, and pro-gastrin-releasing peptide] were each within the normal range. In the specimen taken during the subsequent duodenectomy and remnant gastrectomy, the tumor lesion remained grossly indistinct, like that in the gastrectomy specimen (Fig. 2A). In this second specimen, histology revealed the tumor was located in the duodenum alone. The tumor size (*i.e.*, its extent through the duodenum and gastric antrum) was estimated from the first- and second-operation specimens to be approximately 107×65 mm. Because the tumor had greater density in the duodenum, and because mucosal involve-

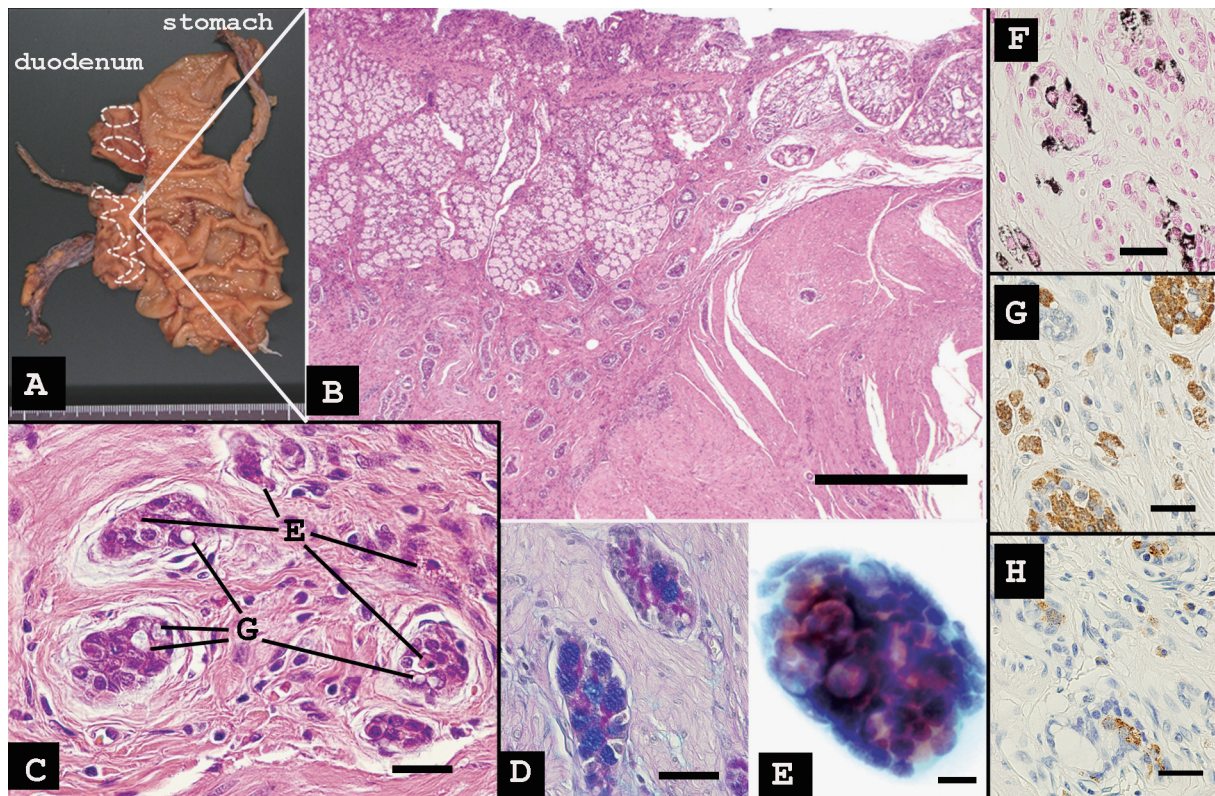


Fig. 2 A, Broken white lines on the duodenectomy and remnant gastrectomy specimen (from the second operation) indicate the region of the tumor. Solid violet line indicates line of anastomosis; B, C, Histology of the main tumor revealed that solid tumor nests, which were distributed throughout the duodenal wall, were covered by eroded mucosa (B). Solid tumor nests infiltrated the duodenal wall with stromal desmoplasia, and some tumor cells displayed goblet cell (G) or eosinophilic granular (E) features (C) (hematoxylin-eosin); D, Mucin deposited within tumor cytoplasm (PAS-Alcian blue double stain); E, Imprint cytology of the tumor revealed a cohesive cell cluster with mucus deposits (Papanicolaou stain); F-H, Neuroendocrine features were highlighted both by the Fontana-Masson argentaffin reaction (F, black as positive) and by immunohistochemical markers for serotonin (G) and somatostatin (H). (G, H; diaminobenzidine). Scale bars in B-H indicate 1 mm, 50 μ m, 50 μ m, 15 μ m, 40 μ m, 40 μ m, and 40 μ m, respectively.

ment (deep layer) was observed in the duodenum alone, the tumor was suspected to have a duodenal origin from which it had extended into the gastric antrum. The tumor cells formed small solid clusters, trabeculae, or tubules, and infiltrated into the duodenal and gastric wall with a stromal desmoplastic reaction. The tumor cells extended throughout the deep mucosa to the subserosa of the duodenum, and were overlaid by non-neoplastic mucosal surfaces (Fig. 2B). The tumor cells possessed large, rounded nuclei, and some displayed an eosinophilic granular cytoplasm or goblet cell features, as revealed by the periodic-acid Schiff reaction or Alcian blue staining, respectively (Fig. 2C, D). Cytologic examination of an imprint from the tumor revealed tumor cell clusters with fine nuclear chromatin patterns and mucinous materials (Fig. 2E). Both argyrophil and argentaffin reactions were positive (Fig. 2F). Immunohistochemical examination yielded positive results for antibodies against epithelial membrane antigen (clone E29; Dako, Glostrup, Denmark), chromogranin A (polyclone; Dako), synaptophysin (polyclone; Cell Marque, Rocklin, CA, USA), serotonin (polyclone; Covance, Princeton, NJ; Fig. 2G), and somatostatin (polyclone; Nichirei Bioscience, Tokyo, Japan; Fig. 2H), although the results were negative for gastrin (clone 4C7A1; MBL, Nagoya, Japan). There was partial expression of CEA (clone II-7; Dako) in the goblet cell component. Electron microscopy revealed that some tumor cells included intracytoplasmic neuroendocrine granules and mucus granules simultaneously (amphicrine cells; Fig. 3). These findings are similar to those reported for appendiceal GCC [2, 3]. Although nuclear pleomorphism was indistinct, mitotic figures were evident, as was perineural and lymphovascular invasion. Carcinomatous infiltration by tumor cells was predominant, especially at the tumor periphery (mostly in the gastric antrum). Some 12–15% of tumor cells were immunoreactive for p53 (clone DO-7; Leica Microsystems, Wetzlar, Germany), and the Ki-67 (clone MIB-1; Dako) index was calculated to be 19%. No tumor deposits were found within the dissected hepatoduodenal or retropancreatic lymph nodes. No other neuroendocrine lesions, such as endocrine cell micronests or hyperplasia, were evident in either the first- or second-operation specimens.

Our patient's post-operative course was uneventful. He was treated adjuvantly with an antineoplastic drug,

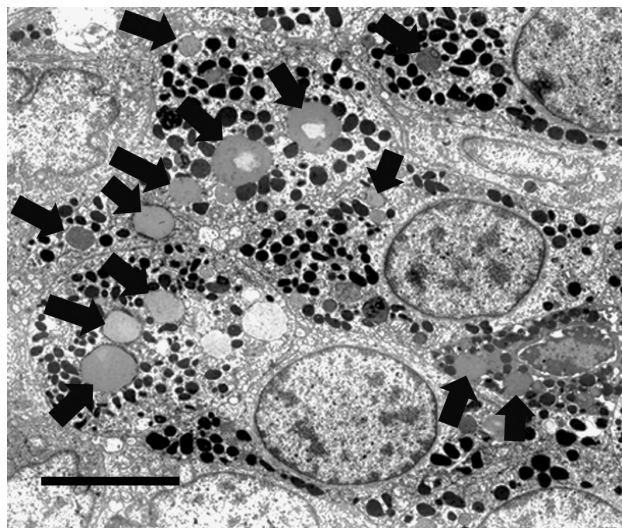


Fig. 3 Transmission electron microscopy revealed simultaneous deposition of dense neuroendocrine granules (smaller granules) and mucus granules (larger ones indicated by black arrows) inside a given tumor cell (JEM-1011, JEOL Ltd., Tokyo; stained with uranyl acetate and lead citrate). Scale bar indicates 10 μ m.

Tegafur-Gimeracil-Oteracil potassium compound, because the tumor showed perineural and lymphovascular invasion, and tumor cell nests extended even to the distal stump of the second-operation specimen. A whole-body investigation by F-18 fluorodeoxyglucose-positron emission tomography/computed tomography 3 months after the second operation did not reveal intense isotope uptake anywhere in his body. Laboratory data for each of the tumor markers CEA, CA19-9, and serotonin remained within the normal range during the post-operative course. Fortunately, he has remained free of signs of tumor recurrence after 2 years of follow-up, although we decided that careful observation should be continued.

Discussion

In cases with a duodenal endocrine tumor, symptoms such as obstructive jaundice, pancreatitis, hemorrhage, intestinal obstruction, and/or gastroduodenal ulcer formation are generally caused either via local infiltration or via secretion of a peptide hormone [1]. In the present case, multiple ulcerative and erosive lesions were observed in both the stomach and the duodenum. Although multiple ulcers (our initial diagnosis) are often caused by overproduction of gas-

trin, as in Zollinger-Ellison syndrome, in the present case the tumor did not produce gastrin. The present tumor was widely distributed in both the duodenum and the gastric antrum, although mucosal involvement was observed only in the first portion of the duodenum. The gastric ulcers, which were distant from the tumor, were considered peptic ulcers, and although a duodenal ulcer seemed a likely cause of the tumor, we could not find one by endoscopic biopsy. These findings are similar to those reported for the discovery of duodenal endocrine tumors by pathologic examination of gastrectomy specimens and of pancreaticoduodenectomy specimens removed for stomach or pancreatobiliary cancers [1]. Therefore, the diagnosis of GCC will depend upon careful clinical and pathologic examinations.

In the present case, the tumor was characterized firstly by such histologic findings as a brightly eosinophilic granular cytoplasm (suggesting the presence of serotonin granules), somatostatin immunoreactivity, and an argentaffin silver impregnation reaction. Somatostatin cell tumors commonly occur at or near the ampulla of Vater in the duodenum [1], whereas the presence of an argentaffin silver impregnation reaction suggested that the tumor shared the features of those in a group of "midgut" enterochromaffin cell (EC cell) carcinoids. Such EC cell carcinoids usually occur in the "midgut" organs (namely, the lower jejunum, ileum, cecum, and appendix), and they are rare in the duodenum (one of the "foregut" organs), although those in the duodenum are considered indistinguishable, both pathologically and behaviorally, from EC cell tumors of the "midgut" [4].

Secondly, the present case was characterized by the presence of glandular differentiation (so-called GCC) features alongside the EC cell differentiation mentioned above. GCC is most commonly found in the appendix (a "midgut" organ). Only 2 cases have been reported within the duodenum [5, 6], both of which presented with gastric outlet stricture or bile-duct obstruction. GCC has been suggested to be a neoplasm composed of cells showing a divergent lineage differentiation from normal crypt stem cells [7]. The immunohistochemical results obtained for the present tumor are compatible with those obtained from appendiceal GCC [8]. However, the appendix had been insignificant during the present course, so the possibility of a metastatic tumor with an appendiceal origin

was ruled out in the present case. The ultrastructural appearance of amphoteric features supports a biphenotypic character for this tumor.

Although the prognosis of duodenal GCC is unclear because of its rarity, we can approach this issue by considering what is known about the prognosis of EC cell tumors and appendiceal GCC. In the case of appendiceal EC cell tumors, metastasis is uncommon and, if it occurs at all, usually involves only the regional lymph nodes [8]. Concerning the 5-year-survival of patients with appendiceal carcinoids (including GCC), this evidently depends on its spread: 94% for patients with localized disease, 85% for regional disease, and 34% for distant metastases [9]. In another report, 12.5% of patients with appendiceal GCCs died as a result of it [2]. Actually, the prognosis would be expected to be intermediate between that of patients with well-differentiated endocrine tumors and those with adenocarcinomas [4]. The markers of an unfavorable prognosis for appendiceal GCCs are considered to be the following: 1) perineural or lymphatic invasion, 2) nuclear pleomorphism, 3) a higher mitotic rate, and 4) a carcinomatous growth pattern in over 50% of the tumor [7]. In the present case, the histology matched at least 2 of the above criteria, and the relatively high value of the Ki-67 index (19%) also suggested an unfavorable prognosis. Nevertheless, our patient has been free from signs of tumor recurrence for 2 years.

In conclusion, we present here a rare case of a duodenal GCC that was not found preoperatively. In addition to a goblet cell appearance of some cells, the tumor exhibited neuroendocrine features such as "midgut" EC cell differentiation and serotonin and somatostatin immunopositivity. Ultrastructural confirmation of its amphoteric nature suggested a tumor having mixed neuroendocrine and exocrine differentiations. Although some findings implied that the tumor might be aggressive, and although a careful follow-up observation period will be required, our patient has been free from signs of tumor recurrence for 2 years. Finally, physicians will probably encounter GCC in the duodenum only rarely, and its discovery is likely to be incidental. Its diagnosis will therefore rely upon careful clinical and pathologic examinations.

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