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Case Report

A Case of Mixed Adenoneuroendocrine Carcinoma of the Stomach With Focal Intestinal Metaplasia and Hypergastrinemia

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Among neuroendocrine neoplasms, mixed exocrine and endocrine characteristics with at least 30% of each component are classified into mixed adenoneuroendocrine carcinoma (MANEC), according to the 2010 World Health Organization classification. We experienced a rare case of MANEC of the stomach with focal intestinal metaplasia and hypergastrinemia. A 76-year-old Japanese male was diagnosed as having gastric adenocarcinoma and underwent total gastrectomy. The pathologic diagnosis was MANEC of the stomach accompanied by unusual mucosal atrophy without *Helicobacter pylori* infection, the characteristics of which were different from both type A and type B atrophic gastritis. The patient has a history of long-term use of a proton pump inhibitor. Additional serum chemistry examination using preoperatively obtained plasma from the patient revealed hypergastrinemia. The mechanism of gastric MANEC carcinogenesis is still unclear, but that might be correlated with unusual intestinal metaplasia and hypergastrinemia in this case.

Key words: Mixed adenoneuroendocrine carcinoma – Stomach – Intestinal metaplasia – Hypergastrinemia – Proton pump inhibitor

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Gastrointestinal neuroendocrine tumors (NETs) constitute a heterogeneous group of tumors originated from neuroendocrine cells in the gut.[1] Mixed adenoneuroendocrine carcinomas (MANECs) show each component of exocrine carcinomas and endocrine carcinomas existing in at least 30% of the tumor, and classified into high-grade malignancies of NETs in the 2010 WHO classification.[2] NETs of the stomach were classified into 3 types, according to the background mucosa.[3] NET type 1 occurs in coincidence with type A chronic atrophic gastritis, as single or multiple small tumors. NET type 2 develops in the patients of the multiple endocrine neoplasia type 1 syndrome (MEN 1) with hypergastrinemia. NET types 3 sporadically develop without any specific conditions. However, the mechanism of gastric NETs tumorigenesis was not fully clarified especially in the cases of MANEC.

Chronic gastritis can be caused by many pathologic factors such as *Helicobacter pylori (H. pylori)* infection, autoimmune disease, and lymphocytic and eosinophilic gastritis.[4] Chronic gastritis is usually divided into type-A and -B. Type-A gastritis is characterized by diffuse atrophic changes of the gastric body and fundus with intestinal metaplasia and inflammatory cell infiltration, and associated with achlorhydria and pernicious anemia.[5] The autoantibodies against parietal cells, proton pump (H^+, K^+-ATPase) and intrinsic factors are considered to play the main pathogenetic roles.[6] On the other hand, type-B gastritis shows mucosal atrophic changes and inflammatory cell infiltration in the gastric antrum.[7] *H. pylori* infection is thought to be an agent of type-B gastritis.[8–10] Gastric carcinogenesis may be promoted by *H. pylori*-positive gastritis.[11]

Here, we report the case of gastric MANEC which developed in the patient with a long term use of proton pump inhibitor (PPI) and hypergastrinemia. The underground gastric mucosa of the patient showed strange atrophic change, which was different from characteristics of type-A and type-B gastritis, whereas enterochromaffin-like (ECL) cell hyperplasia and endocrine cell micronests (ECMs) were seen in fundic gland mucosa.

Case Report

A 76-year-old man presented to our hospital for the diagnosis of early gastric cancer. His past medical history was rheumatoid arthritis and hypertension. He had not underwent any surgical treatment and denied a family history of cancer and any genetic disorders. A proton pump inhibitor and an oral prednisolone at a dose of 5 mg/day had been taken for over 10 years. Physical examination revealed no abnormalities in the abdomen. The blood test data on admission showed 14.7 g/dL at the hemoglobin count and 9.2 mg/dL at the serum calcium level without abnormal serum levels of carcinoembryonic antigen and CA19-9. The patient's serum gastrin was 430 pg/mL. The patient underwent upper endoscopy which revealed a type 0-IIc (superficial depressed) lesion in the posterior wall of the middle body. The tumor biopsy specimen confirmed poorly differentiated adenocarcinoma containing signet-ring cells. The patient underwent a computed tomography (CT) scan of the thorax and abdomen that demonstrated no mass of the stomach and no evidence of distant metastases. No masses of the thyroid or parathyroid were revealed by the CT scan of his neck.

The patient was clinically diagnosed with early gastric cancer and subsequently underwent total gastrectomy, omentectomy, regional lymph node dissection, and Roux-en-Y reconstruction. Intraoperative findings revealed no tumor invasion into the stomach serosa and no evidence of distant metastases. Postoperative course was uneventful. The patient was discharged on the 11th postoperative day. The patient's serum gastrin decreased to normal level (95 pg/mL) 23 days after the surgical resection.

The resected tissues were fixed in 20% formalin. Macroscopically, a 0-IIc lesion measuring 22 × 22 mm was located in the posterior wall of the middle body. The mucosal findings of the nonneoplastic region were unusual, because mucosal atrophic change was observed only in the anterior wall, lesser curvature, and posterior wall of the body, but not in the antrum, greater curvature, and fundus of the stomach. The 0-IIc depressed lesion was located within the atrophic lesion.

The sectioned tissues were stained with hematoxylin and eosin (HE) and Periodic Acid-Schiff (PAS). Immunohistochemical staining for synaptophysin, chromogranin A, Ki-67, MUC5AC, HIK1083, MUC2, and CDX-2 was performed.

Microscopically, 2 distinct neoplastic phenotypes were identified in the tumor (Fig. 1). The proliferation of signet-ring cell carcinoma, which had mucus positive for PAS inside the cell, was found in the surface of the mucosal layer. The other phenotype was composed of smaller cells located in the lower mucosal layer and the submucosal layer, which formed discrete islands in a restiform to

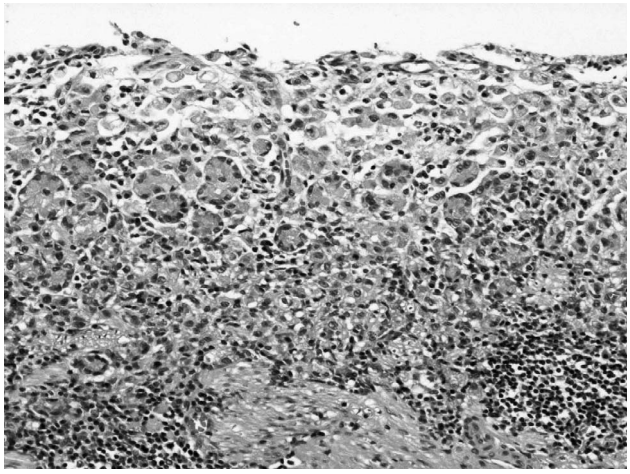

Fig. 1 The tumor cells composed of signet-ring cell carcinoma in the surface of the mucosal layer and of smaller cells located in the lower mucosal layer and the submucosal layer.

alveolar arrangement, which resembled a carcinoid tumor. However, some latter component also contained mucin in the cytoplasm, thus both components were not obviously distinctive especially in the submucosal layer. No evidence of metastatic carcinoma was found in the resected lymph nodes.

Immunohistochemically, the expressions of synaptophysin and chromogranin A were observed in the carcinoid-like tumor cells in the lower mucosal layer and some of tumor cells in the submucosal layer (Fig. 2), which indicated neuroendocrine differentiation of the tumor cells, but not in the signet-ring cells in the upper mucosal layer. Both signet-ring cell and neuroendocrine tumor cells occupied more than 30% of the tumor each. From these findings, we diagnosed the tumor as MANEC of the stomach according to the 2010 WHO classification. Signet-ring cells were positive for gastric foveolar mucin (MUC5AC), and some tumor cells were positive for gastric gland mucin (HIK1083) and intestinal mucin (MUC2). Almost all tumor cells showed strong nuclear CDX-2 expression. The rates of Ki-67-positive proliferating cells were much higher in the signet-ring cell carcinoma component than in the neuroendocrine cell component.

Addition to carcinoma, synaptophysin positive ECL cell hyperplasia, and parietal cell hyperplasia were observed in some gastric fundic glands adjacent to the tumor (Fig. 3). Furthermore, ECMs were detected at the fundic gland base and within the muscular layer of mucosa adjacent to carcinoma (Fig. 4).

Intestinal metaplasia with eosinophil and lymphoplasmacytic cell infiltration was noted in the middle body of the stomach, where macroscopic mucosal atrophy was seen. Carcinoma was located at the atrophic-non atrophic border. No atrophic mucosal change was found in the pyloric part, the upper body, and the fornix of the stomach. The *H. pylori* infection was not detected by immunohistochemical examination. These macroscopic and histologic findings were different from those of type-A and type-B gastritis.

Discussion

NETs are classified into G1, G2, G3 (neuroendocrine carcinoma), and MANEC in the 2010 WHO classification.[12] The histologic grading system from G1 to G3 represents the malignant nature of these tumors.[13–14] The grading system of NETs is based on mitotic rate and/or Ki-67 labeling index, as proposed by the European Neuroendocrine Tumour Society.[13–14] To date, the treatment of patients diagnosed with NETs is decided according to the histopathologic grading of the tumor. However, in spite of a wide range of diverse malignant potentials in each case, MANEC is not subdivided according to tumor grades in the present classification. Recently, La Rossa *et al* proposed the subcategorization of MANEC, in which MANEC is subdivided into high-grade, which consisted of adenoma/adenocarcino-

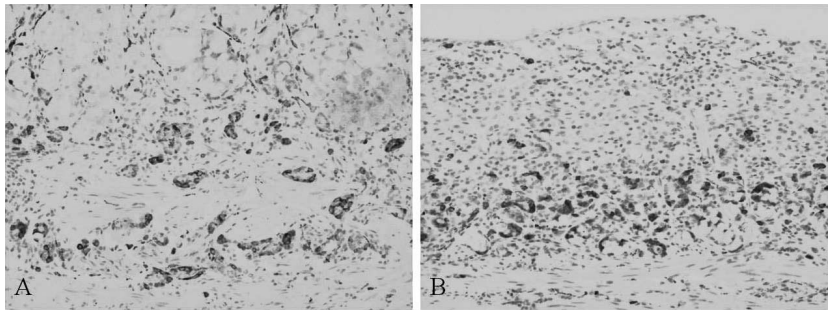

Fig. 2 Synaptophysin (A) and chromogranin A (B) were expressed in the carcinoid-like tumor cells.

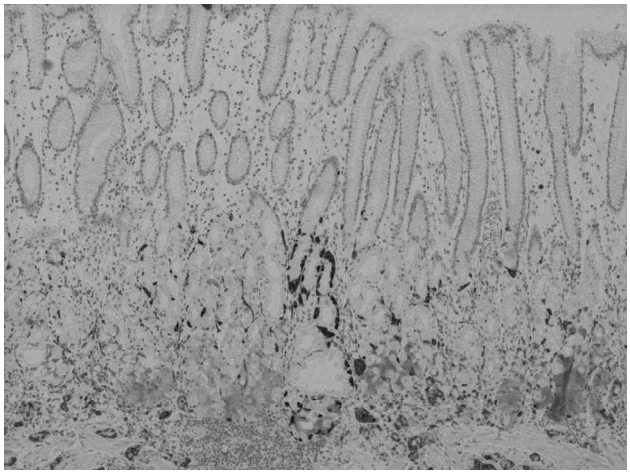

Fig. 3 The hyperplasia of ECL cells positive for synaptophysin was observed in some gastric fundic glands close to the tumor.

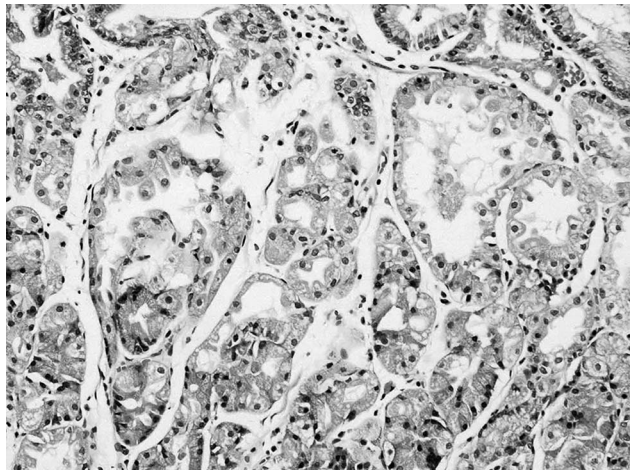

Fig. 4 ECMs were demonstrated at the fundic gland base and within the muscular layer of mucosa adjacent to carcinoma.

ma and neuroendocrine carcinoma; intermediate-grade malignant MANEC, which consisted of adenocarcinoma and G1/G2 NET or amphicrine carcinoma; and mixed adenoendocrine tumor, which consisted of adenoma-NET.[15]

In our case, Ki-67 labeling index in signet-ring cell carcinoma area was more than 80%, whereas that is under 20% in neuroendocrine tumor components. Thus, our case would be classified into intermediate-grade MANEC by La Rossa classification. In previous reports, composite tumors with signet-ring cell carcinoma and neuroendocrine cell tumor seem to have a better prognosis than common gastric adenocarcinomas.[16–17] Our patient did not receive any adjuvant chemotherapy after the operation.

As to the pathogenesis of gastric NETs, NET G1 and G2 are usually subdivided into 3 types of NETs.[3] Type I is the most common, and the tumor usually occurs in the background of autoimmune chronic atrophic gastritis. Autoimmune atrophic gastritis known as type-A gastritis is also a risk factor for gastric cancer.[18] This type of gastric NETs has a tendency of multiple occurring, mainly in the gastric corpus and fundus,[19–20] and often associates with ECMs and ECL cell hyperplasia.[21] The type II tumor occurs in association with hypergastrinemia by Zollinger-Ellison syndrome/multiple endocrine neoplasia type 1 (MEN 1). It has been well known that ECL cell and parietal cell bear gastrin receptor and those with hyperplasia may be induced by hypergastrinemia, which would lead to development of type I and type II NET.[22–23] The type III tumor occurred sporadically and solitarily on the mild mucosal atrophy. These tumors have no association with hypergastrinemia.

On the other hand, carcinogenesis of the neuroendocrine carcinoma (NEC) and MANEC has not been elucidated. The detail of correlation between those tumors and type-A and type-B gastritis or Zollinger-Ellison syndrome/MEN 1 has not been reported. However, Bakkelund *et al* have reported that signet-ring cell carcinomas may originate from the dedifferentiation from ECL cells through signet-ring cells with neuroendocrine immunoreactivity.[24] In our case, MANEC consisted of both signet-ring cell carcinoma and neuroendocrine cell tumor, and ECL cell hyperplasia and ECM were observed in adjacent mucosa. These findings may have supported the hypothesis that a single common precursor cell could develop into mixed glandular-endocrine cell carcinoma of the stomach.[25]

The distribution of gastric mucosal atrophy and intestinal metaplasia in our patient was restricted in the middle and lower gastric body, but not in the upper body and fornix. That is quite different from that of type-A and type-B gastritis. Moreover, our patient has no clinical findings of the existence of gastrinoma and/or MEN 1. The patient's serum gastrin level immediately decreased to normal level after surgery. However, some background findings such as intestinal metaplasia, ECL cell hyperplasia, parietal cell hyperplasia, ECM, and hypergastrinemia seemed to be similar to those of type I or type II NET.

It is controversial whether long-term hypergastrinemia induced by PPI leads to ECL cell neoplasia.[26] Systematic review by Eslami *et al* recently concluded that long-term PPI use did not statistically increase gastric atrophic changes or ECL cell hyperplasia.[27] Differently from the case of type I and type II NET, in which serum gastrin often increase greater than 500 pg/mL,[28–29] PPI do not usually increase serum gastrin more than 300 pg/mL,[30] and PPI is not thought to induce NET. However, Freston *et al* reported that serum gastrin level >400 pg/mL may be caused in about 2% patients treated with long-term use of PPI, while >500 pg/mL was found in less than 1% of patients.[31] Our patient had received a proton pump inhibitor and an oral prednisolone over 10 years before the admission to our hospital and the patient's serum gastrin was 430 pg/mL. Although significant growth of endocrine cell in the stomach nor occurrence of dysplasia or neoplasia has not been reported in their report,[31] it might be possible that hypergastrinemia induced by long-term use of PPI might cause unusual intestinal metaplasia, ECL cell and parietal cell hyperplasia, ECM and MANEC in our patient. It should be necessary to study the relationship between hypergastrinemia and changes of gastric mucosa in a rare case of patients having long-term PPI use.

In conclusion, we reported a rare case of MANEC of the stomach with the partial mucosal atrophy restricted to the middle body of the stomach. The characteristics of the atrophic gastritis were considered to neither type A nor type B gastritis. Further studies are needed to determine the influence of long-term PPI use on the development of MANEC, especially on the patients with severe hypergastrinemia.

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