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CLINICAL CASE SEMINAR

Carcinogenic Hypergastrinemia: Signet-Ring Cell Carcinoma in a Patient with Multiple Endocrine Neoplasia Type 1 with Zollinger-Ellison's Syndrome

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Context: Gastric neuroendocrine tumors are rare neoplasms that originate from gastric enterochromaffin-like (ECL) cells in the oxyntic mucosa. Gastrin and its derivates have been reported to regulate epithelial cell proliferation, migration, and differentiation. Mutations in the epithelial cadherin (E-cadherin) gene have been shown to be associated with the occurrence of diffuse gastric carcinomas in affected families.

Objective: In this study we investigated the histopathological and molecular findings in the gastrointestinal wall of a patient with multiple endocrine neoplasia type 1 with malignant duodenal gastrinoma and multiple gastric ECL cell tumors, who additionally developed a signet-ring cell carcinoma of the stomach.

Design and Patient: Biopsies from the gastrointestinal tract of a patient with multiple endocrine neoplasia type 1 were immunostained for vesicular monoamine transporter-2 and E-cadherin. Nonamidated gastrin products were measured in the serum of the patient using antibodies that react with progastrin, Gly-extended, and amidated gastrins. Genetic analyses were performed to exclude germ-line mutations within the E-cadherin gene.

Results: Immunohistochemical studies of gastric ECL cell tumors showed a largely diminished E-cadherin expression in comparison to gastric surface mucosa cells and a loss of E-cadherin expression in the cells of the signet-ring carcinoma. Detailed biochemical measurements revealed progastrin concentrations that were approximately 20%, and Gly-gastrin concentrations that were approximately 10% the amidated gastrin concentrations in plasma. Molecular analyses revealed no E-cadherin germ-line mutation.

Conclusion: Our immunohistochemical studies might suggest that the gastrinoma-associated excessive progastrin tissue concentrations led to diminished expression of E-cadherin within the gastric mucosa and promoted tumor development of a signet-ring cell carcinoma. (***J Clin Endocrinol Metab* 92: 3378–3382, 2007**)

GASTRIC NEUROENDOCRINE tumors are rare neoplasms that originate from gastric enterochromaffin-like (ECL) cells in the oxyntic mucosa (1, 2). Gastric carcinoids account for less than 1% of all gastric neoplasms and about 9% of all gastrointestinal carcinoid tumors (1, 3). There are four types of gastric carcinoid tumors: type I (70–80%) is associated with chronic atrophic gastritis, type II (5–6%) develops in patients with combined multiple endocrine neoplasia type 1 (MEN1) and the Zollinger-Ellison's syndrome, type III (14–25%) is thought to occur sporadically, and type IV is a poorly differentiated neuroendocrine cancer (1–4). Well-differentiated tumors generally originate from ECL cells. Although the pathogenesis of these tumors is not completely understood, hypergastrinemia plays an important role in the development of type I and II neoplasms (4). Surgical or endoscopic removal of the tumors is recommended in all four types, depending mainly on tumor size. However, these tumors may also be treated with somatostatin analogs (5).

Recently, it was demonstrated that early gastric cancer of the young with signet-ring cell infiltrates is caused by germline truncating mutations in the epithelial cadherin (E-cadherin) gene (*CDH1*), which are supposed to be associated with an impaired function of the E-cadherin protein (6, 7). E-cadherin is a transmembrane protein that forms, together with its counterpart β-catenin, adherens junctions between cells (8). Abnormal expression of β-catenin promotes tumor development in adenomatous polyposis coli mutant mice, suggesting that modulation of adherens junctions may also influence tumor development in different human malignancies (9, 10). Although poorly understood, the establishment and stability of adherens junctions are tightly regulated by growth factors, cytokines, and hormones such as gastrin (11, 12). It has long been known that gastrin is both a growth

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Abbreviations: E-cadherin, Epithelial cadherin; ECL, enterochromaffin-like; MEN1, multiple endocrine neoplasia type 1; VMAT, vesicular monoamine transporter.

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factor and an acid secretagogue. Recently, roles for biosynthetic intermediates, the nonamidated gastrins (progastrin and Gly-gastrin) as cocarcinogens have been described (11). Interestingly, progastrin-derived peptides, including amidated gastrins, may also regulate epithelial cell adhesion or migration. Thus, Gly-gastrin induces the dissociation of the E-cadherin/β-catenin complex, the delocalization of β-catenin from the adherens junctions to the cytoplasm, and the migration of gastric epithelial cells (13). Most recently, it has also been demonstrated that gastrin and amidated gastrin may activate the expression of cyclin D1 through β-catenin in gastric cancer cells and, thereby, inducing gastric cancer cell proliferation (14). In addition, cyclin D1 was up-regulated in signet ring carcinoma cells (15).

In the current paper, we describe a patient with MEN1 with Zollinger-Ellison's syndrome, who in addition developed a signet-ring cell carcinoma of the stomach. Our immunohistochemical and molecular analyses propose the concept of a (pro-)gastrin-induced pathogenesis of the development of signet ring cells via the E-cadherin/β-catenin system.

Patient and Methods

Patient

A 60-yr-old man was admitted to our outpatient clinic in September 1997 with suspected MEN1 syndrome combining primary hyperparathyroidism and Zollinger-Ellison's syndrome with hypergastrinemia. Primary hyperparathyroidism was diagnosed due to elevated serum parathyroid hormone concentration (132 pg/ml, normal range <55), and elevated serum calcium (2.74 mmol/liter adjusted to protein levels) and low serum phosphate (0.72 mmol/liter). Moreover, a parathyroid gland could be detected on ultrasound as well as a positive Sesta-2-methoxy isobutyl isonitrile scan. Zollinger-Ellison's syndrome with hypergastrinemia was diagnosed via positive secretin testing (fasting serum gastrin levels 1610 pg/ml; normal, less than 115; 10 min after application of 120 U secretin gastrin levels increased up to 2350 pg/ml). Genetic screening showed a heterozygous mutation in the *Menin* gene (chromosome 11, exon 2, codon 104; deletion of thymidine). At that time the patient refused any further diagnostic investigations or therapeutic interventions.

In July 2002, he again presented to our outpatient clinic with epigastric pain, which had been ameliorated after oral treatment with omeprazole (30 mg/d). Serum gastrin level was increased approximately 30-fold (3380 pg/ml) before treatment with omeprazole, whereas chromogranin A was increased approximately 4000-fold (445,000 μg/liter; normal < 110). On computed tomography neither tumor-suspect lesions nor pathological lymph node swellings were seen.

Gastroduodenoscopy revealed multiple polypoid gastric tumors (Fig. 1, B–E) and a duodenal tumor (Fig. 1A). Histology of gastric biopsy sections revealed typical features of multifocal gastric ECL cell tumors that stained strongly with antibodies against vesicular monoamine transporter (VMAT)-2 (Fig. 2) and chromogranin A. Micronodular and linear ECL cell hyperplasia was also present. However, unexpectedly, diffuse signet-ring cell infiltrations negative for the neuroendocrine markers synaptophysin and chromogranin A, as well as VMAT-2, were also seen (Fig. 2). Because the patient still refused any surgical intervention, we initiated a therapy with a somatostatin analog for the treatment of the Zollinger-Ellison syndrome and carcinoid tumors (Sandostatin LAR 30 mg every 4 wk; Novartis International AG, Basel, Switzerland), as previously described (5).

Two follow-up gastroscopies 4 and 9 months later revealed a similar picture of a polypoid gastric mucosa. Additional biopsies taken at three different sites (duodenal bulb, antrum, and corpus) again confirmed the diagnosis of a signet-ring cell carcinoma. At that time serum gastrin levels decreased to 2155 pg/ml and chromogranin A to 59,600 μg/liter, which was most likely due to our treatment with the octreotide receptor analog. Eighteen months after initial diagnosis, the patient presented to our clinic with malignant ascites caused by peritoneal signet-ring cell infiltrates. Despite initiation of ip (mitoxantrone) and systemic chemotherapy (etoposide, folinic acid, and fluorouracil), the patient died during follow-up.

Immunohistochemistry

Serial paraffin-embedded sections were stained with a polyclonal rabbit antibody against human gastrin (code no. A 0568; dilution 1:6000; DakoCytomation, Hamburg, Germany), VMAT-2 (rabbit polyclonal code no. AB1767; dilution 1:200; Chemicon International, Inc., Temecula, CA) synaptophysin, chromogranin-A, and a mouse monoclonal antibody against E-cadherin (product code NCL-E-Cad; dilution 1:100; Novocastra, Dossenheim, Germany) in a moist chamber at 37 C for 60 min. Bound antibody was detected using the avidin-biotin complex peroxidase method (ABC Elite Kit; Vector Laboratories, Burlingame, CA). The staining reaction was performed with 3,3′-diaminobenzidine and H_2O_2 and counterstaining with hematoxylin.

Genetic analysis

DNA was extracted from the peripheral blood by phenol-chloroform extraction. Mutation analysis was performed as previously described (16). In doing so, single-strand conformational polymorphism and heteroduplex analyses were applied. The 16 exons of the E-cadherin gene were amplified in fragments of 200–400 bp. PCR primers were used as reported previously (16). Genomic DNA was amplified under standard PCR conditions. Reaction products were diluted 1:1 with a denaturing buffer and heated to 99 C for 10 min before loading onto 0.8× mutation detection enhancement gels (Flowgen Bioscience Ltd., Nottingham, Nottinghamshire, UK), both with and without 10% glycerol. Gels were run for 12–18 h at 10 C and stained with silver nitrate.

In addition, direct DNA sequencing was performed. The entire E-cadherin coding sequence and intron-exon boundaries were sequenced. PCR products were purified from 2% low-melting point agarose (Life Technologies, Inc., Gaithersburg, MD) using Wizard purification columns (Promega, Bad Naunheim, Germany). Purified products were sequenced on an ABI373 automated sequencer (Applied Biosystems, Foster City, CA) using TaqFS cycle sequencing.

RIA analysis of progastrin-derived peptides

Progastrin-derived peptides in the serum of the patient were measured by using antibodies, to progastrin (L289), Gly-extended gastrin (109–21), and amidated gastrin (L2), as published previously (17).

Results

Histopathology

Histology of biopsies that were taken from the gastric antrum and corpus revealed typical features of type 2 gastric ECL cell tumors that showed strong immunoreactivity for VMAT-2 (Fig. 2). Biopsies of the duodenal tumor were strongly positive for gastrin representing the potential source of Zollinger-Ellison's syndrome in this patient. However, strikingly, within the gastric biopsies, diffuse signet-ring cell infiltrations were also seen, which were negative for endocrine markers such as synaptophysin, chromogranin-A. and VMAT-2 (Fig. 2). The proliferation index of signet ring cells was about 5%. The gastric ECL cell tumors showed a largely diminished E-cadherin expression when compared with the gastric surface mucosa and the signet-ring cell carcinoma was E-cadherin negative (Fig. 2).

Measurement of serum gastrin derivates

Detailed biochemical measurements in our patient's case revealed progastrin concentrations that were approximately 20% (between 0.60 and 0.70 nM) and Gly-gastrin concentra-

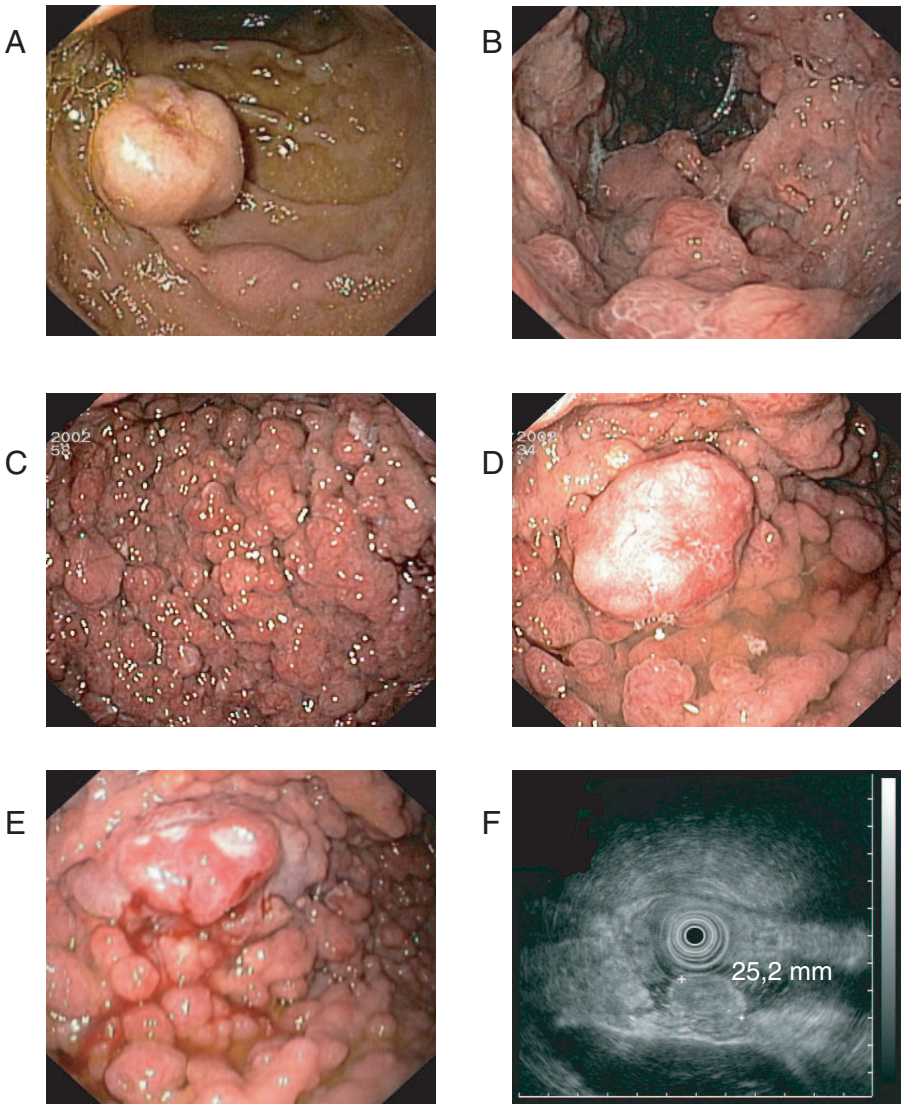

FIG. 1. Endoscopy shows a duodenal tumor (A) as well as diffusely spread gastric tumors (B–E) with polypoid growth. Biopsies were taken from the duodenal tumor and different sites of the stomach (E). On endosonography of the stomach, tumors within the gastric wall were detected with up to 25 mm in maximum size (F).

tions that were approximately 10% (between 0.28 and 0.36 nM) the amidated gastrin concentrations (between 3.6 and 4.5 nM) in the patient's plasma.

Molecular analyses

To exclude germ-line mutations within the E-cadherin gene, we also performed a mutation analysis of the whole gene. However, no abnormalities were identified. This is in accordance with the observation of strong E-cadherin expression on the gastric surface.

Discussion

Here, we describe a patient with Zollinger-Ellison's syndrome with highly elevated serum gastrin levels due to a MEN1-associated duodenal gastrinoma, who additionally developed a gastric signet-ring cell carcinoma and metastatic spread. There are different hypotheses with respect to the pathogenetic mechanism underlying the development of a signet-ring cell carcinoma in this MEN1 patient. One explanation would be that the signet ring cells developed coincidentally; another could be that this carcinoma occurred due to the impaired menin function because of the MEN1 germ-line mutation. An alternative third explanation would focus on the downregulation of the E-cadherin expression and liberation of β-catenin from these complexes due to the influence of (pro-) gastrin and its derivates. Our immunohistochemical and molecular studies support this hypothesis as follows.

E-cadherin is a transmembrane protein with five tandem repeated, extracellular domains and a cytoplasmatic domain that forms the adherens junctions to the actin cytoskeleton through a complex with α-, β-, and γ-catenins (8). Diminished E-cadherin expression is associated with aggressive, poorly differentiated carcinomas (18, 19). Underexpression of E-cadherin is a prognostic marker of poor clinical outcome in many tumor types (9). Restored expression in tumor models can cause growth retardation or decreasing invasiveness in epithelial tumor cells (10, 18, 20). Furthermore, it has been demonstrated in a transgenic mouse cell line that loss of E-cadherin mediated cell adhesion is one of the rate-limiting steps in the progression from adenoma to carcinoma (21).

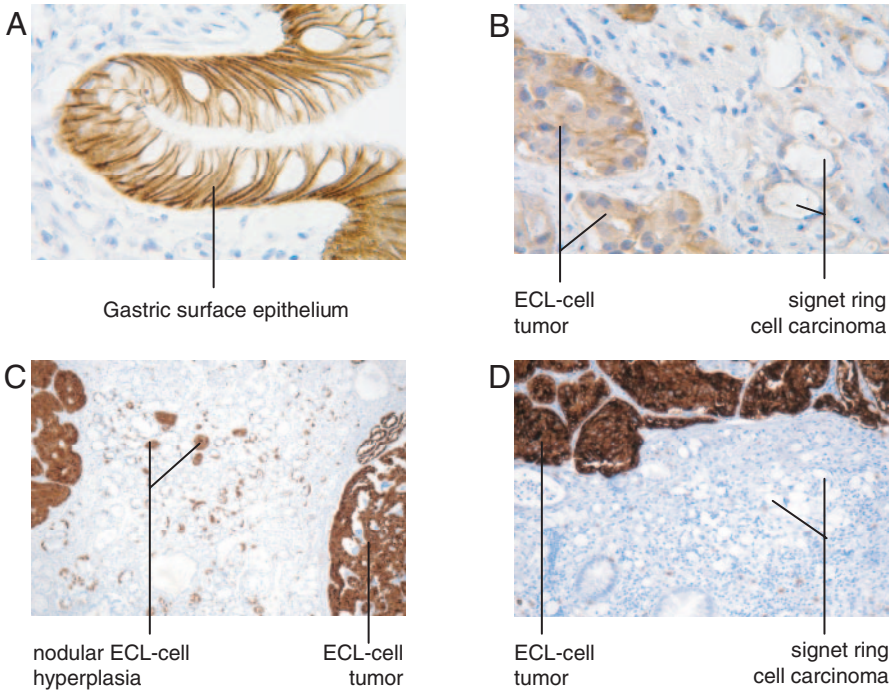

FIG. 2. Representative immunohistochemistry of an endoscopic biopsy from the gastric antrum showing diminished E-cadherin expression of the ECL cell tumor cells and a loss of E-cadherin expression in the cells of the signet-ring carcinoma (B). Unaffected gastric surface epithelium showing strong membranous E-cadherin expression (A). Representative immunohistochemistry of gastric antrum showing VMAT-2 positive ECL cell tumors and nodular ECL cell hyperplasia (C), as well as a VMAT-2 positive ECL cell tumor and VMAT-2 negative signet-ring cell carcinoma (D).

In humans the importance of this cell adhesion molecule in tumorigenesis had also been established with the first description of a clear molecular basis for familial gastric cancer in three Maori kindreds (6). Inactivation of the second (wild-type) allele in a somatic cell of the gastric mucosa would lead to a total loss of E-cadherin. In case of hereditary diffuse gastric cancer, the wild-type allele could be inactivated by either a point mutation or CDH1-promoter hypermethylation (22). Sporadic invasive lobular breast cancer has also been seen in several of these families (18, 23). Furthermore, in approximately 50% of patients with somatic inactivating CDH1 mutations, the occurrence of poorly differentiated foci in colorectal and prostatic adenocarcinomas was seen (24).

The establishment and stability of the cell-cell adherens junctions between cells formed by E-cadherin and β-catenin are tightly regulated by growth factors, cytokines, and hormones. In the gastrointestinal tract, they are regulated for instance by gastrin and its prohormone progastrin (25, 26). Glycine-extended gastrin induced partial dissociation of the E-cadherin/β-catenin complex via PI3-mediated tyrosine phosphorylation and, thus, migration of gastric epithelial cells (13). It was shown that prolonged moderate hypergastrinemia leads to remodeling of the gastric epithelium and in the presence of *Helicobacter* to gastric cancer (27). In colorectal carcinoma cells, gastrin gene expression is increased by oncogenic mutations in the Ras gene. Recently, in two different mouse models, the observation was made that gastrin plasma levels above the physiological range disrupted the controlling processes of tubulogenesis in gastric glands and accelerated oncogenic progression together with other factors (27). In transgenic mice with hypergastrinemia, there are initially increased parietal cell numbers and increased gland lengths, indicating that gastrin influences the organization of the gastric mucosa (28). Gastrin stimulates the tubulogenesis in gastric AGS cells (cell line from American Tissue Culture Collection; no. CRL-1739) through activation of the cholecystokinin-$_B$-receptor, with high affinity for gastrin, C-terminal amidated gastrins (*e.g.* G17; Fig. 3), and cholecystokinin (29), via protein kinase C. The pathways of MAPKs or PI-3 kinase do not seem to be involved (27). Therefore, gastrin is the first neuroendocrine peptide acting through G protein-coupled receptors that has been shown to be capable of stimulating branching morphogenesis.

Progastrin induced the dissociation of both tight junction and adherens junction complexes in a gastric epithelial cell line as well as cell migration (25). In transgenic mice overexpressing progastrin, there is increased epithelial cell proliferation in the colon, increased thickness of colon mucosa, as well as increased susceptibility to carcinogens (29). The glycine-extended gastrins can act as modulators of parietal cell function by regulating the capacity of cell response to secretagogues (27). Gly-gastrin also

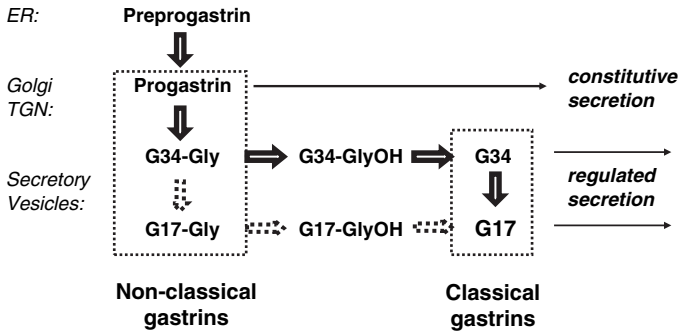

FIG. 3. Biosynthetic relationships of pre-progastrin-derived peptides. Major pathways of processing are indicated by *blocked arrows*, minor pathways by *broken-line arrows*. *Boxes* indicate the "non-classical" and "classical" gastrins. The amidated peptides G34 and G17 are secreted from vesicles of the regulated pathway of exocytosis that may also contain some unprocessed "non-classical" gastrins; in non-endocrine cells, *e.g.* colorectal carcinoma, progastrin may be secreted directly from the *trans*-Golgi network (TGN) to the cell surface by the constitutive route.

activates Src tyrosine kinase, and this has been linked to dissociation of tight junctions (25, 29). Furthermore, Gly-gastrin, but not progastrin or amidated gastrin, seems to play a crucial role in bronchoalveolar carcinoma in both transgenic mice and humans (30). There is evidence that a cholecystokinin-$_B$-receptor variant with affinity of Gly-gastrins is expressed in colorectal, pancreatic, and esophageal tumors, but not in normal mucosa (29). Detailed biochemical measurements in our patient's case revealed elevated progastrin concentrations that were approximately 20%, and Gly-gastrin concentrations that were approximately 10% the amidated gastrin in plasma, also supporting this concept that progastrin and its derivates are responsible for tumor development *in vivo*.

In summary, our observations support the hypothesis that progastrin-derived peptides may down-regulate cell-cell adherens junctions and/or activate β-catenin-dependent signaling, thus contributing to tumor development of the signet-ring cell carcinoma. A germ-line mutation leading to the loss of the E-cadherin gene expression on a molecular level could be excluded in our patient's case. This is supported by the fact that gastric surface epithelium showed strong E-cadherin expression. Therefore, the development of a signet ring carcinoma may be due to increased pro- and Gly-gastrin expression. As suggested in other studies (5), life-long therapy with somatostatin analogs may represent an alternative treatment for patients with hereditary gastric/duodenal Zollinger-Ellison's syndrome. However, in such patients regular endoscopic biopsies appear to be mandatory, also to exclude the formation of a signet ring-cell carcinoma.

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