Review

Gastrointestinal Endocrine Tumors: Recent Developments

Cesare Bordi, MD, Tiziana D'Adda, BSc, Cinzia Azzoni, BSc, Gabriella Canavese, MD, and Maria Luisa Brandi, MD

Abstract

Endocrine tumors of the gastrointestinal tract are a heterogeneous population of neoplasms differing from each other by their secretory and functional properties, pathogenetic mechanisms, related clinical syndromes, pathological associations, and prognosis. Although such heterogeneity and the relative rarity of these tumors hamper systematic investigations, significant advances in several aspects of tumor pathology have been achieved in recent years. This article focuses on the genetic background of tumor development with emphasis on the role of the MEN-1 oncosuppressor gene in either MEN-1-associated or sporadic endocrine tumors, on the identification and pathologic relevance of tumor-produced growth factors, and on the mechanism of induction of gastrin-dependent ECL cell carcinoids of the stomach.

Key Words: Carcinoid tumors; gastrinomas; MEN-1 syndrome; MEN-1 gene; host responses to tumors; growth factors; ECL cell; BCL-2; α-subunit of human chorionic gonadotropin.

Introduction

The gastrointestinal mucosa encompasses at least 12 different types of endocrine cells [1]. For unknown reasons, more than half of these cells do not give rise to tumors at all or do it exceptionally. Even so, the endocrine tumors of the gastrointestinal tract represent a heterogeneous population of neoplasms (Table 1) differing from each other for their secretory and functional properties, pathogenetic mechanisms, related clinical syndromes, pathological associations, and prognosis. Furthermore, this heterogeneity involves also tumors originating from the same cell type as testified by the three types of ECL cell carcinoids in the stomach or by the EC cell carcinoids in the ileum and cecum or in the appendix.

Unfortunately, these concepts are scarcely recognized in clinical and biological studies dealing with gastrointestinal endocrine tumors that are often based on heterogeneous cumulated series, thus yielding results often contradictory and difficult to interpret. The overall small number of these tumors, owing to their low incidence, amplifies the problems connected with their cytological subdivision. In spite of these difficulties, in recent years, important developments have been achieved in our knowledge of the biological and molecular mechanisms driving development and progression of gastrointestinal endocrine tumors as well as their influence on the tissue microenvironment. In particular, this article will focus on the expression of MEN-1 gene, the role of growth factors, and the mechanism of induction of gastrin dependent gastric ECL cell carcinoids.

Department of Anatomic Pathology, University of Parma, Parma, Department of Clinical Physiopathology, Endocrine Unit University of Florence, Florence, Italy.

Address correspondence to: Cesare Bordi, MD, Istituto di Anatomia Patologica, Università di Parma, I-43100 Parma, Italy. E-mail: bordi@ipruniv.cce.unipr.it

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Table 1. Defined Types of Gastrointestinal Endocrine Tumors[a]

Tumor types	Cell of origin	Main hormone production
Stomach		
Carcinoid tumor type I	ECL	Histamine
Carcinoid tumor type II	ECL	Histamine
Carcinoid tumor type III	ECL	Histamine
Gastrinoma	G	Gastrin
Neuroendocrine carcinoma	Unknown (stem?)	None
Duodenum		
Gastrinoma	G	Gastrin
Somatostatinoma	D	Somatostatin
Gangliocytic paraganglioma	?	Somatostatin, PP
Carcinoid tumor	EC	Serotonin
Neuroendocrine carcinoma	Unknown (stem?)	None
Small intestine and cecum		
Carcinoid tumor	EC	Serotonin
Rectum and sigmoid colon		
Carcinoid tumor	L	Enteroglucagon, PP, PYY
Neuroendocrine carcinoma	Unknown (stem?)	None
Appendix		
Carcinoid tumor	EC	Serotonin

[a]Mixed exocrine-endocrine tumors are not considered. Occasional examples of EC cell carcinoids may also be found in the stomach and colon-rectum, of somatostatinomas in the small and large bowel, and of L cell carcinoids in the small bowel-cecum and appendix.

Classification of Gastrointestinal Endocrine Tumors

Table 1 lists the gastrointestinal endocrine tumors for which adequate descriptions are available. A detailed classification of these tumors is beyond the scope of this article, and the reader is referred to several excellent reviews or books [2–4]. For the reader's convenience, the fundamental data of those tumors that are further discussed in this article are described hereafter.

Gastric ECL Cell Carcinoids

Three independent types of these tumors have been identified on the basis of the associated pathological conditions:

1. Type I, associated with atrophic corporal gastritis (ACG). It accounts for 70–80% of all gastric (ECL cell) carcinoids. ACG, depending on either autoimmune condition (pernicious anemia) or Helicobacter pylori infection [5], and the related achlorhydria is the causative condition for secondary hypergastrinemia of antral origin that is consistently associated with these tumors and represents a potent trophic stimulus for ECL cell proliferation [6]. The tumors more frequently affect the female gender (71% of cases). Macroscopically, they are more frequently multiple, usually appearing as small, clinically silent polyps that tend to be circumscribed to the mucosa or, most often, to the submucosa. Histologically, the neoplasms show a typical carcinoid structure and are consistently associated with proliferation of extratumoral ECL cells from which the tumors are presumed to originate through a sequence of hyperplasia-dysplasia-neoplasia. Metastases to regional lymph nodes are rare (<5%) and to distant sites exceptional. No cases of tumor-related death are on record [7].
2. Type II, associated with multiple endocrine neoplasia type 1 (MEN-1) and, usually, Zollinger-Ellison syndrome (ZES). It accounts for about 6% of ECL cell carcinoids and shows no gender prevalence. The tumors are usually multiple and, though larger than those of type I, are smaller than 1.5 cm in size in 73% of cases [7]. Histologically, type II carcinoids are similar to type I tumors, including the associated feature of precursor lesions of ECL cells in extratumoral mucosa. Tumor infiltration does not extend beyond the submucosa in 91% of cases, whereas metastases to lymph node are present in 30% of patients [7]. The prognosis of these tumors is usually good, but cases with a very aggressive course or association with highly malignant neuroendocrine gastric carcinomas have been seen recently [8].
3. Type III or sporadic, not associated with hypergastrinemia or other significant pathological conditions of the stomach.

It accounts for 14–25% of all ECL cell carcinoids and shows a striking predominance for the male gender (74%) [7]. The tumors are usually single and in 33% of cases larger than 2 cm in diameter. Infiltration of the muscularis propria is found in 76% and of the serosa in 53% of cases. Although most neoplasms histologically are typical carcinoids, tumors with more atypical appearance are often found, especially when exceeding 2 cm in size [9]. Metastases, often involving the liver, occur in three-fourth of patients, mostly with atypical carcinoids. Tumor-related death is seen in 27% of cases with a median survival of 28 mo [7].

Duodenal Gastrinomas

They account for about two-thirds of all duodenal neuroendocrine tumors, are preferentially located in the proximal duodenum, and are usually small (<1 cm in diameter) [4]. Their histological appearance is typical. Metastases are common, even in the smallest tumors, but usually confined to regional lymph node(s). Most of the so-called primary nodal gastrinomas are considered to be metastases from occult duodenal neoplasms. Liver metastases are rare and, usually, late events. Duodenal gastrinomas may be either sporadic or associated to MEN-1 syndrome roughly in a ratio of 2:1. As a rule, MEN-1 tumors are multicentric and may escape detection even at surgery [10].

Midgut (Ileum, Cecum) EC Cell Carcinoids

They account for the vast majority of neuroendocrine tumors of the ileum and cecum, and are characterized by their immunohistochemical expression of serotonin and substance P. At the time of diagnosis, these tumors are usually larger than 1 cm in size, multiple in up to 40% of cases, and tend to infiltrate the muscular wall and to give metastases to lymph nodes [4]. Their histological structure is typical, showing solid clusters arranged in an insular pattern. Proliferation of EC cells in contiguous crypts suggests precursor changes [11]. A carcinoid syndrome is apparent in about 20% of cases and is strictly dependent on the establishment of liver metastases.

Appendix EC Cell Carcinoids

They account for the overwhelming majority of appendicular neuroendocrine tumors, and exhibit the same histological appearance and immunohistochemical expression of their midgut counterpart. In contrast, they appear to originate from submucosal neuroendocrine complexes being closely associated with Schwann-like, S-100 immunoreactive, sustentacular cells [4,11]. The tumor tend to be small, discovered serendipitously, not associated with the carcinoid syndrome, and with almost invariably benign course even if infiltration of the muscularis propria is common. Only tumors larger than 2 cm in size or infiltrating the mesoappendix are potentially metastasizing.

Rectal L Cell Carcinoids

These tumors are usually found to produce peptides of the glucagon-glicentin and of the PP-PYY families [12], being in this respect fully different from those of the remaining large bowel, which mostly produce serotonin and are composed of EC cells. They tend to be small, asymptomatic, often polypoid neoplasms, expanding in the submucosa and showing a distinctive trabecular structure.

Neuroendocrine Carcinomas

These are highly malignant tumors composed of small to intermediate-sized cells

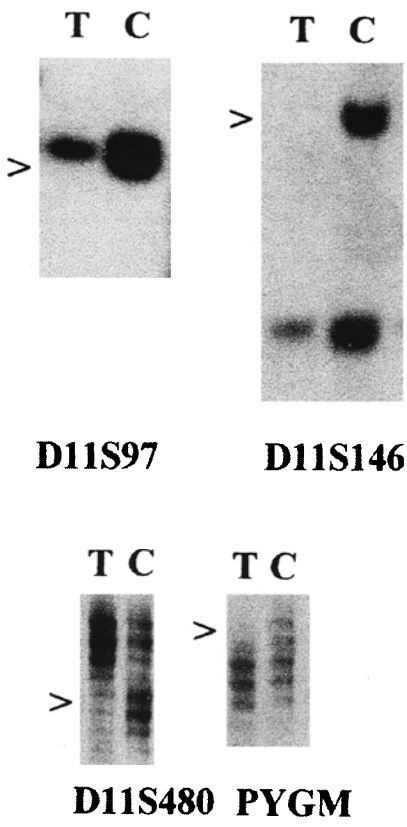

Fig. 1. Allelic loss at the 11q13 region in a gastric carcinoid tumor (upper panel) and in a gastric neuroendocrine carcinoma (lower panel) in two MEN-1 patients shown by microsatellite-PCR analysis. The polymorphic DNA markers used for the analysis are reported below each autoradiogram. Arrows indicate allelic loss in tumor DNA (lane T) in comparison to constitutive DNA (lane C). From Bordi et al. [8].

reminiscent of the small-cell carcinoma of the lung. Their neuroendocrine nature is usually validated by immunostaining for nongranular neuroendocrine markers, such as neuron-specific enolase, synaptophysin, or PGP 9.5, whereas granular markers, such as chromogranins, are scarcely reactive or completely unreactive. They are more frequently located in the stomach, the ampullary region, and the colon-rectum. As discussed later, neuroendocrine carcinomas may either derive from dedifferentiation of differentiated endocrine tumors, or arise *de novo*. Occasionally, association with MEN-1 syndrome has been reported [8]. Their outcome usually is ominous.

Expression of the MEN-1 Gene

Two members of the family of gastrointestinal endocrine tumors, the type II ECL cell gastric carcinoid and the duodenal gastrinoma, are integral components of the MEN-1 syndrome [13], suggesting an involvement of the MEN-1 gene in their development.

The MEN-1 gene has been located on chromosome 11q13 and found to act as a tumor suppressor gene [14]. In affected patients, the genotype of MEN-1 tumors is assumed to consist of an inherited germline mutation of the MEN-1 gene and loss of function of the wild-type allele through chromosomal deletion (loss of heterozygosity, LOH) or point mutations, the inactivation of the wild-type copy of the gene representing the transforming factor for tumor development according to Knudson's two-hit theory. Cloning and sequencing of the MEN-1 gene are very recent [15]. Thus, adequate information on the genotype/phenotype correlations is not yet available. However, large amount of data have been generated on 11q13 LOH using polymorphic genetic markers closely linked to the MEN-1 gene. Various patterns of deletion have been detected in 63–100% of MEN-1 parathyroid tumors and in 83–92% of pancreatic endocrine tumors, but gastrinomas [16].

LOH at 11q13 of type II gastric carcinoids was found in 9 of 10 MEN-1 patients investigated [8,17–19] (Fig. 1). These results strongly support the notion that these carcinoids are integral components of the MEN-1 phenotype sharing with parathyroid and nongastrinoma islet-cell tumors the highest frequency of LOH

at 11q13 among MEN-1 neoplasms. When multiple carcinoids from the same patient were examined, the deletion size in the wild-type allele proved to differ from one tumor to another, indicating independent somatic events in separate lesions [19]. In one case, only three of eight concomitant carcinoids examined showed allelic loss, suggesting that more subtle defects, such as small interstitial deletions or point mutations, may be responsible for the somatic inactivation of the wild-type allele in some tumors [19]. In all cases but one, the patients were also affected by ZES. Thus, in these cases, the promoting effect of hypergastrinemia in the sequence ECL cell hyperplasia-dysplasia-neoplasia likely is operating [6,20]. Of interest is the observation of the remaining case showing multiple ECL cell neuroendocrine carcinomas and benign carcinoids with LOH at 11q13 in the absence of hypergastrinemia and/or gastrinomas. This case demonstrate that inactivation of MEN-1 gene *per se* is an adequate oncogenic stimulus independent of the trophic effect of hypergastrinemia [8].

The role of the MEN-1 gene in non-MEN-1 gastric carcinoids is more controversial. In a study of six type I gastric carcinoids, Debelenko et al. found 11q13 LOH in only one tumor [19]. In contrast, D'Adda et al. (in preparation) in a study of 25 tumors from 15 patients found 11q13 LOH involving at least two genetic markers in 12 (48%). In 4 patients, multiple tumors were investigated, accounting for a total of 14 carcinoids, and in all of them, tumors with deletions coexisted with tumors without demonstrable deletion, again indicating independent tumorigenic events in separate neoplasms. No LOH at 11q13 was found in three type III gastric carcinoids investigated so far (D'Adda et al., in preparation). Whether these findings indicate a different pathogenesis for sporadic, type III gastric carcinoids deserve to be ascertained in more extensive studies.

Duodenal gastrinomas may be either associated with MEN-1 syndrome or sporadic. Surprisingly, MEN-1 gastrinomas were found to be associated with a rather low incidence of allelic loss at the MEN-1 gene locus. In a study of 34 tumors, deletions were found in only 14 of them (41%) [21], a figure remarkably lower than that shown by parathyroid, pancreatic, and gastric MEN-1 neoplasms. This finding suggests that for unknown reasons, somatic inactivation of the wild-type allele by small deletions or point mutations is the most common mechanism for development of MEN-1-associated duodenal gastrinomas. Alternatively, further genetic alterations are required in these neoplasms in addition to the initial inactivation of the MEN-1 gene [21]. In the same study, LOH was found in three of six sporadic duodenal gastrinomas, a figure similar to that of MEN-1-associated tumors. In both MEN-1-associated and sporadic tumors, no differences in size and histology were found between neoplasms with and without deletions [16].

Involvement of MEN-1 gene in carcinoids of mid/hindgut derivation is more problematic. These tumors are not associated with the MEN-1 syndrome and a study of nine cases (one jejunum, five ileum, one appendix, two rectum) using at least three informative polymorphic markers in each case revealed no allelic loss at 11q13 [19]. In contrast, another study showed LOH in eight of nine tumors (three ileum, three appendix, and three colon) [22]. In the latter study, however, deletions were often multiple and discontinuous, and were also found in the short arm of chromosome 11, suggesting involvement of genes other than MEN-1. Mutational analysis of the recently cloned MEN-1 gene is crucial to clarify these issues. Likely, knowledge of the molecular

mechanisms controlling the MEN-1 gene expression and the function of the related menin protein will further contribute to clarify the still unanswered questions.

Studies on other genes potentially involved in the genesis of gastrointestinal endocrine tumors have been unsuccessful so far. The genes more commonly implicated in human cancerogenesis do not appear to be significantly involved in the development of differentiated gastrointestinal endocrine tumors. In particular, p53 point mutation was found in only one, clinically benign rectal carcinoid out of a large, representative series of 33 carcinoids from all gastrointestinal locations [23] and 1 of 15 midgut, EC cell carcinoids [24]. Moreover, no *ras* mutations were detected in a series of 22 carcinoids from both the duodenum and the midgut [25].

Most poorly differentiated neuroendocrine carcinomas appear to develop on a different genetic background. p53 mutations involving exons 6–8 were found in 4 of 5 such neoplasms in the midgut regions [24]. A study of colorectal neuroendocrine carcinomas from nine non-MEN-1 patients revealed LOH of the genes associated with the genesis of colorectal nonendocrine adenocarcinomas (APP, DCC, p53) [26]. When neuroendocrine carcinomas were associated with ordinary adenocarcinomas in the same patients, an identical pattern of allelic loss was found in the two tumor components, indicating that at least in colorectal mucosa, both types of neoplasms derive from the same cell of origin through a similar cancerogenetic pathway. In contrast, extensive LOH at 11q13 was found in one neuroendocrine carcinoma developed in the gastric body of an MEN-1 patient [8] (Fig. 1), thus indicating a pathogenetic mechanism similar to that of MEN-1 gastric carcinoids. These discordant results suggest a dual origin of neuroendocrine carcinomas either as *de novo* development from undifferentiated stem cells common to nonendocrine carcinomas or as dedifferentiation of pre-existing differentiated endocrine tumors.

Growth Factors (GFs)

GFs are polypeptides that interact with specific cell receptors to produce a spectrum of biological responses, mostly but not exclusively cell proliferation and differentiation [27]. At least seven families of GFs have been identified [28]. Several characteristics differentiate GFs from common hormones: they are produced by a variety of cell types, not exclusively endocrine, usually have a short distance action, also related to their short half-life, and their receptors are promiscuous, having the ability of binding an entire family of GFs [28]. They also have autocrine properties playing an essential role in tumor development and progression [27].

Pathological Evidence for a Role of GFs in Gastrointestinal Carcinoids

A wide spectrum of proliferations of local host tissues consistent with a functional response to tumor production and release of GFs has been found in several types of gastrointestinal carcinoid tumors.

The serotonin-producing EC cell carcinoids of the midgut are the tumor type most frequently involved in this condition. In addition to the well-recognized proliferation of subendocardial fibrous tissue in the right heart leading to the cardiomyopathy that is an integral part of the carcinoid syndrome [29], these tumors have been found to induce proliferation of tumor-associated fibroblasts, smooth muscle cells, blood vessels, vascular elastic tissue, and nerves. The considerable fibroblastic response elicited by the tumor

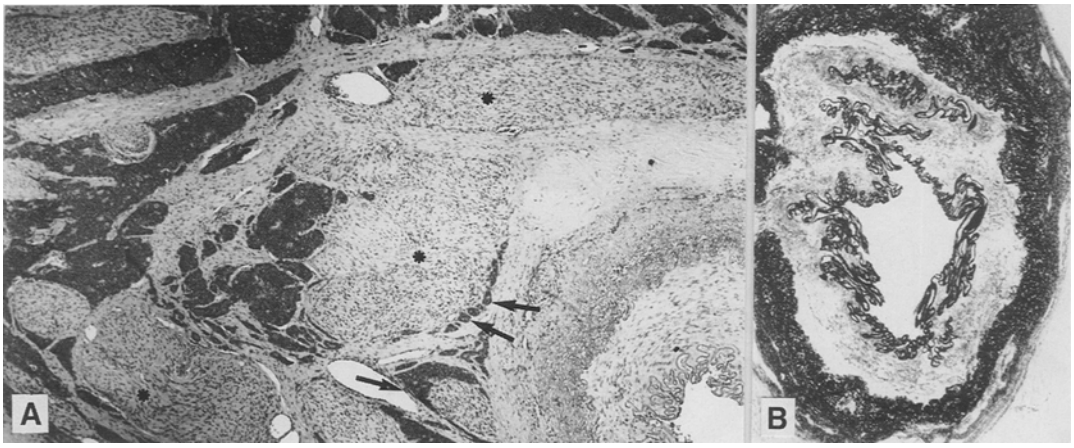

Fig. 2. (A) Marked hypertrophy of nerves (*) running along an artery of the mesenteric root diffusely infiltrated by an EC cell ileal carcinoid. Perineural tumor infiltration is pointed out by arrows (H & E staining, ×42,5). **(B)** Marked vascular elastosis in the same artery shown in panel A demonstrated by Weigert's elastin stain (×31).

infiltration of the mesentery and peritoneum frequently results in buckling on the mesenteric border of the bowel wall, leading to kinking of the bowel and, eventually, to intestinal obstruction [30]. As originally described by McNeal [31], the muscle layers of the ileal wall, in particular of the inner one, show hypertrophy sharply limited to the area of tumor penetration. Diffuse proliferation of both intimal and adventitial elastic tissue in large arterial and venous vessels of the mesenteric pedicle, often called vascular elastosis (Fig. 2), is a specific feature of ileal and jejunal carcinoids, absent in hindgut carcinoids [32,33]. It is sometimes associated with proliferation of extravascular elastic tissue fibers within the bowel wall [33]. Frequently vascular elastosis may cause ischemic changes or even intestinal infarction. Although this complication is usually associated with extensive tumor infiltration of perivascular tissues [32,33], it may also occur as an early presentation of a small, initially infiltrating neoplasm [34]. Hypertrophy of mesenteric nerves running in areas of severe tumor infiltration may also be found (Fig. 2). Finally, prominent proliferation of small vessels and associated myofibroblastic cells causing enlargement of the villi has been reported in the ileal mucosa surrounding the tumor, but distinct from it, suggesting a field effect of GFs secreted by tumor cells [35].

Several aspects of muscle cell proliferation have been described in the gastric mucosa of patients harboring ECL cell carcinoid tumors [36]. They include:

1. Conspicuous hypertrophy of the muscularis mucosae (MM) infiltrated by the tumors.
2. Proliferation of intratumoral stromal smooth muscle cells originating from the MM and mostly associated with tumor invasion of the submucosa.
3. Increased thickness of the MM in the fundic, but not in the antral mucosa.
4. Occurrence of frequent, prominent aggregates of smooth muscle cells in the lamina propria of the antral (but not of the fundic) mucosa of the stomach.

With the exception of the latter finding, the close anatomical relationship of these muscle cell proliferations with carcinoid tumors (as well as with precursor lesions [36,37]) is suggestive of ECL cell production of GFs. A similar mechanism

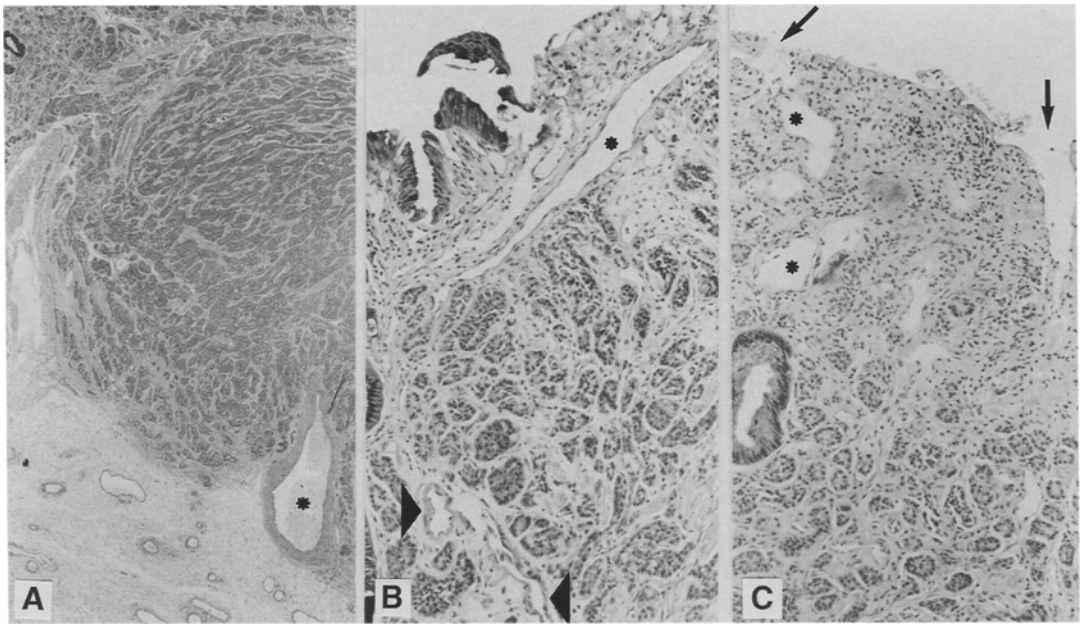

Fig. 3. Anomalous vascular findings in a type II gastric carcinoid causing severe, life-threatening bleeding. **(A)** Abnormally large artery (*) penetrating the lower border of the carcinoid tumor infiltrating the submucosa. In serial sections, this artery can be followed up to the muscularis mucosae. (PAS stain, ×45). **(B)** Abnormally large arterioles (arrowheads) and a large sinusoid space (*) underlying the superficial epithelium in a mucosal region infiltrated by the tumor. **(C)** Opening of dilated sinusoids (*) on the denuded mucosal surface (arrows) (B,C: H & E staining, ×110). Modified from Roncoroni et al. [38].

has also been proposed to explain vascular malformation and/or proliferation that has been found in gastric carcinoids and that may cause severe, life-threatening gastric hemorrhage [38] (Fig. 3).

Association of neuromuscular hyperplasia with carcinoid tumors of the appendix was originally described by Masson [39]. Also in this condition, a potential paracrine influence of extraepithelial neuroendocrine cells and carcinoid cell clusters has been suggested [40]. It is noteworthy that local responses of host tissues similar to those found in gastrointestinal endocrine tumors have not been reported so far in pancreatic endocrine tumors.

GFs Identified in Gastrointestinal Carcinoids

Transforming Growth Factor-α (TGF-α)

It is a 50 amino acid polypeptide that binds to epidermal growth factor (EGF) receptor and stimulates cell growth. Using immunocytochemistry and/or Northern blot analysis, TGF-α and EGF receptors were found to be extensively produced by GI carcinoid tumors, a property shared by other extraintestinal neuroendocrine tumors [41,42]. The effectiveness of the EGF receptors of tumor cell to respond to the TGF-α autocrine stimulus was documented in vitro [42]. TGF-α was ubiquitously expressed by different types of GI carcinoids, including those of the stomach (ECL cell), duodenum (somatostatin cell), ileum and appendix (EC cell), and rectum (L cell) [43]. Its expression was found to be unrelated to the tumor size or to the occurrence of metastases, but was more frequent in tumors infiltrating the muscularis propria than in those limited to the mucosa/submucosa, suggesting a role for local invasion [43].

Transforming Growth Factor-β (TGF-β)

Naturally occurring in three isoforms (β_1, β_2, β_3), TGF-βs are secreted as latent complexes, including the precursor molecule and a latent TGF-β binding protein

(LTBP). TGF-βs have fibrogenetic and angiogenetic effects, and stimulate production of extracellular matrix [41,44]. TGF-β and its mRNA have been consistently found in midgut (EC cell) carcinoids by immunohistochemistry, *in situ* hybridization, and Northern analysis [41,44,45]. The stromal component of the tumors revealed immunohistochemical expression of TGF-β receptor and of LTBP [44]. These results make TGF-β a plausible candidate for the peritumoral and serosal fibroblastic response of midgut EC cell carcinoids. Interestingly, TGF-β (and, in particular, β_1 and β_3) were found to be expressed by fibroblasts in the subendocardial fibrotic plaques of carcinoid heart disease, suggesting a role in the progressive deposition of matrix proteins characteristics of this serious complication of the carcinoid syndrome [46].

Insulin-Like Growth Factors (IGFs)

Two peptides, IGF-1 and IGF-2, have structural homology with proinsulin and biological effects in common with insulin. Their mitogenic activity is mediated by specific, structurally different receptors, labeled as types I and II. IGF-1 was demonstrated by immunocytochemistry in primary cultures of midgut (EC cell) carcinoids and by RIA in tumor extracts of all 11 neoplasms investigated [47]. IGF-1 secretion by cultured tumor cells was inhibited by the somatostatin analog octreotide [45]. IGF-1 receptors were detected immunocytochemically in tumor cells: this observation and the finding of a coexpression of IGF-1 and of the proliferating cell nuclear antigen (PCNA) in subsets of carcinoid tumor cells suggest that autocrine trophic activity is a major function of IGF-1 in carcinoid tumors [45,47]. IGF-1 also has potential neurotrophic effects [45,47].

Platelet-Derived Growth Factor (PDGF)

PDFG is composed of disulfide-bonded A- and B-polypeptide chains, resulting in three dimeric combinations (AA, AB, BB). It bounds to two receptors: α, with high affinity for all isoforms; β, with specific affinity for B-chains. PDGF has mitogenic properties for mesenchymal cells (including fibroblasts and smooth muscle cells) and for neurons [45,48]. Using immunohistochemistry and *in situ* hybridization on frozen tissues, PGDF-B and PGDF-α receptors were found to be expressed by either tumor cells and stroma of most midgut (EC cell) carcinoids, whereas PGDF-A and PGDF-β receptors were preferentially or exclusively demonstrated in the tumor stroma [48]. The occurrence of PGDF-α receptors, which bind to either A- or B-chains, in tumor and stromal cells supports both autocrine stimulation of tumor cells and stromal proliferation in EC cell carcinoid tumors.

Acidic and Basic Fibroblast Growth Factors (aFGF, bFGF)

These are the most extensively studied members of a family of heparin binding peptides. They are widely distributed in the body tissues, and are both known to promote angiogenesis and to induce proliferation of fibroblasts and other mesoderm-derived cells [49]. In addition, aFGF was found to have neurotrophic properties regulating neuronal cell differentiation and survival both in vitro and in vivo [50,51]. On the other hand, a bFGF-like substance is known to be abnormally elevated in the serum of MEN-1 patients [52], largely contributing to the circulating parathyroid mitogenic activity in MEN-1 syndrome [53].

In a detailed immunohistochemical study of 41 endocrine tumors of the whole gastrointestinal tract, La Rosa et al. showed consistent expression of aFGF in seroto-

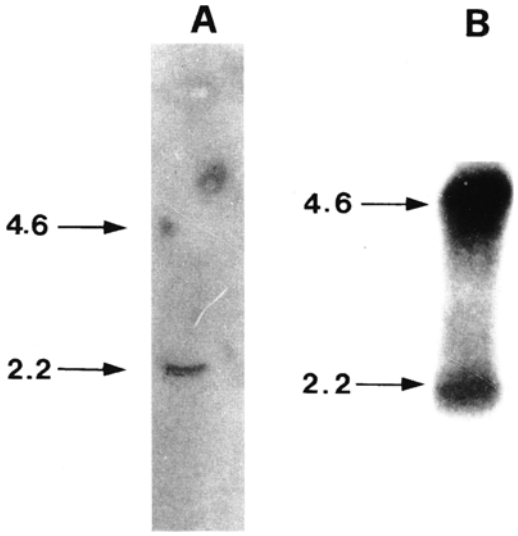

Fig. 4. Northern analysis of tumor tissue from a type I **(A)** and a type II **(B)** ECL cell carcinoid tumors of the stomach. Two major transcripts of approx 4.6 and 2.2 kb, consistent with those of mRNA of human basic fibroblast growth factor, are shown (arrows). From Bordi et al. [55].

nin-producing EC cell carcinoids of the ileum, appendix, right colon, and rectum [54]. The peptide was expressed by a subset of tumor cells, in keeping with the findings of EC cells in the normal intestinal mucosa. In contrast, no aFGF immunoreactivity was found in the other gastrointestinal endocrine tumors investigated. In this study, a correlation was found between the expression of aFGF and the amount of tumor fibrous stroma, supporting a role for this peptide in the tumor-associated fibroblastic response.

Using immunohistochemistry and Northern analysis, we demonstrated that bFGF is expressed by a subset of normal oxyntic endocrine cells and by proliferating ECL cells of hypergastrinemic patients, including both hyperplastic lesions and carcinoid tumors [55] (Fig. 4). The ECL cells, therefore, may represent a potential source of the parathyroid mitogenic factor in patients with MEN-1 syndrome. Indeed, we have found the highest degree of bFGF expression in hyperplastic ECL cells of an MEN-1 patient showing multiple metastasizing ECL cell carcinoids [8] (Fig. 5). It has been suggested that locally released bFGF, a potent mitogen for smooth muscle cells, is the growth factor responsible for the pronounced stromal proliferation of smooth muscle cells frequently associated with ECL cell carcinoid tumors, as previously mentioned, and with their precursor lesions [36].

Immunocytochemical expression of bFGF was also found in some duodenal gastrinomas (Canavese et al., unpublished observations; Fig. 5). Thus far, however, no evidence has been provided for field effects potentially related to tumor GF release in the duodenum. Immunohistochemical results for bFGF in EC cell, midgut carcinoids are conflicting. Using formalin-fixed, paraffin-embedded tissues, La Rosa et al. were unable to find immunoreactivity of tumor cells [54]. In contrast, using frozen, cryosectioned, and acetone-fixed tissues Chaundry et al. reported consistent immunostaining in a large series of tumors [56], whereas immunostaining for bFGF receptor was restricted to tumor stroma. The critical preservation of bFGF antigenicity in routinely processed tissues [55,57] may potentially be responsible for false-negative results [36]. Expression of the four transcripts of bFGF was documented in cell cultures of an EC cell carcinoid [41].

Neuronotrophic Factor(s)

Production of factors stimulating the growth of nerve fibers by gastrointestinal endocrine tumors has been elegantly documented by experimental studies. Transplants of tumor tissue in the anterior eye chamber of immunosuppressed rats elicited growth of sympathetic nerves from the host iris [58,59]. Even more convincingly was the observation that coculture of midgut (EC cell) carcinoid tumor cells and rat

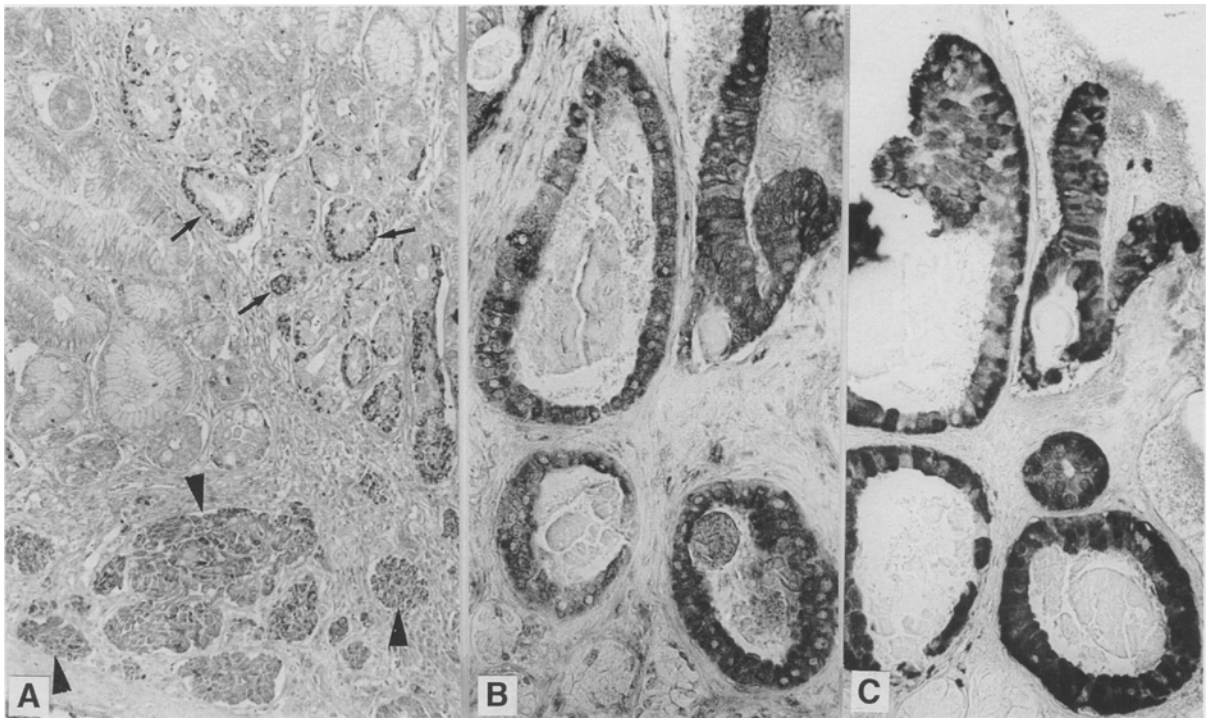

Fig. 5. Immunohistochemical expression of basic fibroblast growth factor. **(A)** Gastric mucosa of a patient with MEN-1 and Zollinger-Ellison syndromes showing intense immunoreactivity in both hyperplastic precursor lesions (arrows) and carcinoid tumors (arrowheads) (×100). Modified from Brandi et al. [13]. **(B)** Immunoreactivity of a duodenal gastrinoma with gland-like histologic arrangement. **(C)** Gastrin immunostaining of the same tumor of panel B in a consecutive section (B,C, ×265).

fetal cholinergic neurons in serum-free media resulted in sustained survival of neurons with pronounced extension of neurites, whereas no neuron survival was obtained in monocultures [60]. The relevant, transferable neurotrophic factor has not been identified yet.

Immunological analogies with nerve growth factor (NGF) were suggested by immunofluorescence staining of cultured carcinoid EC cells [60]. However, Northern blot analysis and specific bioassays proved to be negative for NGF [45]. Among the potential candidates are IGF-1, TGF-α, aFGF, and PDGF, all possessing neuronotrophic effects as discussed above. Receptors for the three former peptides have been demonstrated in neurons [45], whereas PGDF α-receptors were shown by immunocytochemistry in enteric nerve bundles including those close to the carcinoid tumor tissue [48].

Conclusions

Gastrointestinal endocrine tumors have been shown to be active, heterogeneous producers of GFs, providing clues for the interpretation of the conspicuous field responses in the host tissues, sometimes responsible for severe clinical complications. Further investigations in this field may open promising perspectives for elucidating the role of GFs in endocrine tissues.

Mechanism of Induction of Gastrin-Dependent ECL Cell Tumors

The pathogenesis of ECL cell carcinoids arising in hypergastrinemic conditions

(types I and II) has been by far more extensively investigated than that of other endocrine tumors in the gastrointestinal tract and pancreas. This is owing to a number of reasons. First, these tumors develop through a sequence hyperplasia-dysplasia-neoplasia that has been characterized from the histopathological point of view [61] and that can be easily investigated in humans using routine endoscopic gastric biopsies. Second, several experimental models of the disease are available, mostly based on hypergastrinemia secondary to pharmacological inhibition of acid secretion in rodents. They include loxtidine treatment of mastomys [62], which induces ECL cell neoplasms after 12–16 wk in 80% of animals, and treatment of rats with either proton pump inhibitors or H2-receptor blockers [63,64], which in contrast requires lifelong administration of toxicological doses of the drugs. Third, the involvement of drugs largely used in clinical practice has driven huge amount of resources in relevant investigations. As a result substantial information on the mechanism of tumor development has been obtained indicating involvement of both promoting and transforming agents.

Promoting Factors

The potent trophic action of gastrin on the ECL cell in experimental animals and humans has long been known [6,65]. Hypergastrinemic states, depending either on a secondary response of antral G cells to hypo-/achlorhydria or on inappropriate hormone release from gastrinomas, are consistently associated with ECL cell proliferation that is quantitatively related to the circulating gastrin concentrations [6,65]. However, the available clinical evidence indicates that ECL cell exposure to hypergastrinemia alone, a condition occurring in patients with the sporadic form of ZES, does not cause cell transformation with evolution from hyperplasia to neoplasia and that transforming factors must operate in those conditions, such as ACG or MEN-1 syndrome in which the neoplastic evolution is commonly seen.

Experimental observations on the timing of the proliferative response of ECL cells to hypergastrinemia provide a clue for the self-limiting trophic role of gastrin. Studies on thymidine incorporation of ECL cells in omeprazole-treated rats [66] showed a transient peak elevation of the labeling index (LI) restricted to the first 2 wk of exposure to hypergastrinemia. Thereafter, the LI declined and returned to control levels at 10 wk, in spite of persistent hypergastrinemia and elevated concentrations of ECL cells. Experiments with isolated ECL cells from loxtidine-treated mastomys [62] showed that gastrin stimulation of DNA synthesis is 10-fold less potent in proliferated than in normal ECL cells. Thus, the results of both experiments concur in showing that the trophic effect of hypergastrinemia has an independent role only at the very beginning of the proliferative sequence of the ECL cells. The promoting action of hypergastrinemia, however, persists in transformed ECL cells as shown by carcinoid regression after antrectomy, a procedure resulting in normalization of gastrin levels [67].

As discussed in detail elsewhere [68], the female gender has been found to potentiate the trophic effect of gastrin on ECL cells remarkably. In keeping with this assumption is the striking prevalence of female patients in type I, ACG-associated ECL cell carcinoids at variance with an 80% incidence of male patients in type III, gastrin-independent ECL cell carcinoids [9].

Transforming Factors

The role of the MEN-1 gene defects for the progression of ECL cell hyperplasia to

Table 2. Frequency of BCL-2 Immunoreactive ECL Cells[a] in Normal Subjects and in Patients with ECL Cell Hyperplasia (Modified from ref. [70])[b]

Patients		Fasting gastrin, ng/L	BCL-2/CgA immunoreactive cells, %
n	Age		
Subjects with normal serum gastrin and oxyntic mucosa			
10	39 (16–81)	<100	50.0 (24.6–74.0)
Sporadic Zollinger-Ellison syndrome			
9	52 (35–60)	1900 (470–29450)	4.6[c] (0.9–42.0)
Zollinger-Ellison syndrome and multiple endocrine neoplasia type 1			
4	50 (30–61)	570 (560–1000)	55.6 (29.4–83.8)
Corporal atrophic gastritis			
9	50 (22–70)	520 (58–1150)	87.6[d] (12.1–199.4)

[a]Expressed as percentage of chromogranin A (CgA) immunoreactive cells in the same field.
[b]Data are expressed as median (ranges).
[c]Difference from normal: $p < 0.001$.
[d]Difference from normal: $p < 0.006$.

carcinoid tumors has been discussed previously. Inactivation of this suppressor gene appears to be the predominant transforming factor for ECL cell carcinoids developing on the genetic background of the MEN-1 syndrome (type II) [19].

The transforming factor(s) involved in ECL cell tumors associated with ACG (type I) are less clearly defined. Either achlorhydria and related bacterial and/or chemical perturbations of the intragastric environment, or the altered, largely metaplastic mucosal background have been considered [5,6]. Studies from our laboratory indicated a role for BCL-2, a protein encoded by the proto-oncogene bcl-2 that enhances cell survival by blocking programmed cell death (apoptosis). Activation of the bcl-2 oncogene and related BCL-2 overexpression have been documented in a variety of human tumors [69], and contribute to tumor induction and progression by extending cell survival and, therefore, cell exposure to oncogenic factors.

We have found immunohistochemical expression of BCL-2 by hyperplastic ECL cells of hypergastrinemic patients [70]. Interestingly, the degree of such expression correlated with the risk of ECL cell carcinoid development (Table 2). If compared with normal controls, in fact, it appeared to be significantly lower in cases of sporadic ZES (with low or no carcinoid risk), unchanged in cases of ZES/MEN-1 (of intermediate carcinoid risk), and significantly increased in cases of ACG (with the highest incidence of carcinoid development). That the major role of BCL-2 is in the precursor hyperplastic step of tumor induction was indicated by the erratic and, in most cases, weak or absent BCL-2 immunoreactivity of established ECL cell carcinoids.

The abnormal regulation of the bcl-2 gene in hyperplastic ECL cells was found to be independent of the influence of gastrin [70]. Considering the timing of ECL cell response to hypergastrinemia mentioned above, we suggested that BCL-2 overexpression may represent the mechanism that in ACG patients replaces the early, gastrin-dependent peak of mitotic proliferation, thus extending the life-span of formerly proliferated ECL cells, and allowing for the accumulation of genetic and environmental influences necessary for tumor induction [70].

A similar mechanism involving TGF-α has been proposed in the development of ECL cell tumors in the loxtidine-treated mastomys [62]. Using bromo-deoxyuridine uptake in vitro, in fact, Tang et al. found that the TGF-α stimulation of ECL cell DNA synthesis was complementary to that of gastrin, being moderate in normal ECL cells (highly sensitive to gastrin stimulation) and 60-fold higher in hyperplastic ECL cells (scarcely sensitive to the gastrin stimulus) [62]. Furthermore, using RIA

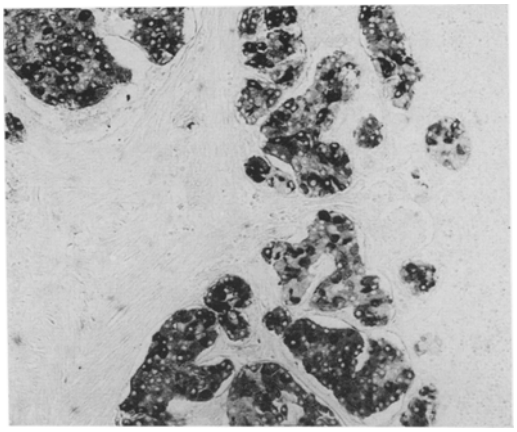

Fig. 6. Unusual, extensive expression of α-subunit of chorionic gonadotropin in a gastric carcinoid of a MEN-1 patient who died from metastatic disease (×110).

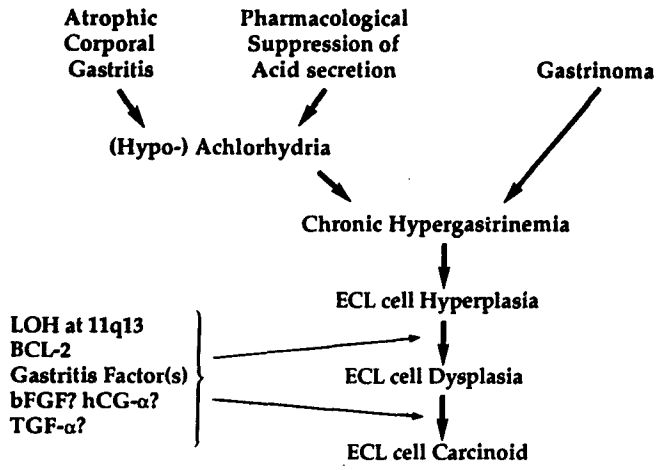

Fig. 7. Schematic outline of the mechanism involved in the development of ECL cell carcinoids in hypergastrinemic patients. Modified from Bordi et al. [6].

and Northern analysis, they also showed that the expression of TGF-α was remarkably higher in hyperplastic than in normal ECL cells and that the expression of ECL cell EGF receptors—specific for TGF-α—presented a pattern parallel to that of TGF-α. TGF-α has not been investigated so far in human ECL cells.

The α-subunit of human chorionic gonadotropin (hCG-α) is another protein that is maximally expressed in stimulated ECL cells of hypergastrinemic patients being virtually undetectable in normogastrinemic subjects [6,71]. As for BCL-2, the expression of hCG-α is significantly reduced in ECL cell carcinoids, suggesting potential implications of the protein for the induction rather than for the progression of these tumors. However, the specific role of hCG-α in ECL cell transformation has not been clarified. Interestingly, the few ECL cell tumors displaying heavy expression of hCG-α (Fig. 6) were found to be associated with unfavorable outcome [8], thus behaving similarly to most hCG-α expressing pancreatic endocrine tumors [72].

Whether bFGF, the other GF identified in ECL cells [55], has an active role in the development of ECL cell carcinoids remains unknown. The schematic outline of the mechanism of ECL cell tumorigenesis in hypergastrinemic conditions is depicted in Fig. 7.

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