Pathogenesis of ECL Cell Tumors in Humans

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In ECL cell tumors developed in the setting of hypergastrinemic conditions (ECL cell carcinoids type 1 and 2), hypergastrinemia is the dominant agent acting as a promoter in all steps (hyperplasia-dysplasia-neoplasia) of the tumorigenic sequence. In contrast, it apparently lacks transforming properties as shown by the absence of ECL cell carcinoids in patients exposed to hypergastrinemia alone, i.e., those with sporadic Zollinger-Ellison syndrome. The potential transforming factors include: the allelic loss of the MEN-1 suppressor gene in the genetically predisposed MEN-1 patients, an alteration that may induce ECL cell tumors even in the absence of hypergastrinemia; the still unknown factor(s) associated with atrophic corporal gastritis; agents whose role in the induction of human ECL cell tumors is still unclarified, such as basic Fibroblast Growth Factor, human Chorionic Gonadotropin-α and Transforming Growth Factor-α; and agents having a favoring role on the ECL exposure to mitogens such as BCL-2. No information is currently available on the pathogenesis of gastrin-independent, sporadic ECL cell carcinoids (type 3) or of gastric neuroendocrine carcinomas.

INTRODUCTION

According to their more recent classification [1-3] human ECL cell tumors (or carcinoids) are subdivided into three types: 1) associated with atrophic corporal gastritis (ACG)[b]; 2) associated with multiple endocrine neoplasia type 1 (MEN-1) and, usually, Zollinger-Ellison syndrome (ZES); and 3) also defined as sporadic, not associated with any significant gastric or endocrinological disease. In addition, ECL cells may also be involved in the development of poorly differentiated neuroendocrine carcinomas, at least in those tumors retaining some characteristics of cell differentiation.

The mechanisms governing the induction of type 3, sporadic ECL cell carcinoids and of gastric neuroendocrine carcinomas are almost completely ignored. In these neoplasms the usual presentation as single tumors, the absence of a background of diffuse hyperplastic and/or dysplastic proliferation of extratumoral ECL cells and the frequent finding of endocrine cell proliferation in the glandular neck epithelium contiguous with the tumor [4] indicate a focal carcinogenetic event in the renewal zone of the oxyntic glands induced by factor(s) still unknown.

This is not the case for type 1 and 2 ECL cell carcinoids. These tumors are often multiple and represent the ultimate event of a widespread proliferation of ECL cells in the oxyntic mucosa [5, 6], features that are consistent with a genetic and/or blood-born nature of the involved oncogenic factors. The occurrence of diffuse precursor lesions and the easy access to their appropriate investigation provided by endoscopic gastric biopsies together with the availability of several experimental models [7, 8] allowed for a substantial progress in our

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[b]*Abbreviations:* ACG, atrophic corporal gastritis; MEN-1, multiple endocrine neoplasia type 1; ZES, Zollinger-Ellison syndrome; LOH, loss of heterozygosity; hCG-α, alpha subunit of human chorionic gonadotropin; bFGF, basic fibroblast growth factor; TGF-α, transforming growth factor-alpha.

understanding of the mechanisms involved in the development of these types of ECL cell neoplasms in humans. This review, therefore, is restricted to type 1 and 2 ECL cell carcinoids and intends to examine the specific factors having a potential role in tumor induction.

HYPERGASTRINEMIA

The potent trophic action of gastrin on the ECL cell is long known [9]. On this basis, hypergastrinemia is the first and most widely recognized factor involved in ECL cell growths of hypergastrinemic patients [5, 6, 10-12]. Thus, the so-called "gastrin concept", formulated to explain the mechanism linking pharmacological inhibition of acid secretion and ECL cell tumor induction in rodents [7], may well be adapted to man. In hypergastrinemic conditions, ECL cell carcinoids develop through a sequence hyperplasia-dysplasia-neoplasia which has been characterized from the histopathological point of view [13]. A threshold value of circulating gastrin is apparently necessary for evoking ECL cell proliferation whereas ECL cell carcinoid tumors do not develop unless gastrin levels exceed 400 ng/L [6, 14]. Decrease and/or normalization of hypergastrinemia by either antrectomy or octreotide treatment induce regression of proliferating ECL cells (Table 1) [15-17] including carcinoid tumors [18]. However, whereas antrectomy specifically inhibits ECL cells, octreotide has a more diffuse inhibitory effect also involving the other types of oxyntic endocrine cells [17].

It is worth noting that among the three main human hypergastrinemic conditions, ACG, MEN-1/ZES, and sporadic ZES, ECL cell carcinoids develop only in the first two. In contrast and with two possible exceptions [19, 20], the gastrin-dependent proliferation of ECL cells does not extend beyond hyperplastic lesions, mostly of the simple and/or linear types [13], in sporadic ZES, an appropriate model for investigating exposure of human ECL cells to hypergastrinemia alone [6]. From this observation it may be inferred that in humans hypergastrinemia acts as an effective promoter for ECL cell proliferation but is unable to induce the transformation of hyperplastic changes of ECL cells into carcinoid tumors. Additional factors associated with ACG or MEN-1 (see below) are therefore necessary for such progression. The promoting action of hypergastrinemia, however, persists in established ECL cell tumors as shown by tumor regression after antrectomy [18, 21].

Table 1. Effect of six months' treatment with octreotide (500 µg/d) on fasting gastrin and on chromogranin A (CgA) and human chorionic gonadotropin-α (hCG-α) immunoreactive cells of the gastric body mucosa (mostly ECL cells) in eight patients with corporal atrophic gastritis (modified from Ferraro et al., 1996).

	Before treatment	After treatment	Difference (percent)	P value
Fasting gastrin (ng/L)	950 (250-1800)	238 (10-825)	-74.9	0.01
CgA immunoreactive cells Volume density (%)*	3.70 (1.82-11.24)	2.10 (1.04-5.10)	-43	0.0131
hCG-α immunoreactive cells Volume density (%)*	0.78 (0-2.23)	0.12 (0-0.41)	-85	0.0007

*The reference volume is the mucosal epithelial component.
Values are expressed as median (ranges).

ATROPHIC CORPORAL GASTRITIS (ACG)

ACG is the pathological condition showing the highest frequency of ECL cell carcinoids (type 1) in humans. With one possible exception [22], hypergastrinemia is a consistent feature of all cases of ACG-associated gastric carcinoids. The transforming mechanism involved in ECL cell carcinoid induction in ACG patients is unknown. Either achlorhydria and related bacterial and/or chemical perturbations of the intragastric environment, or the altered, largely metaplastic mucosal background have been considered [6, 11, 23].

The type of ACG associated with ECL cell carcinoids is generally regarded to correspond to the autoimmune variety, associated with pernicious anemia and restricted to the acid-secreting mucosa, formerly labeled as type A [1, 12, 14, 24]. However, ECL cell carcinoids are increasingly detected also in patients with the multifocal type of ACG, largely caused by *Helicobacter pylori* infection, and recognizable at the histological levels mostly because of the persistence of clusters of oxyntic glands containing parietal cells [23]. The actual incidence of the different types of ACG associated with ECL cell carcinoids, however, has not been investigated yet.

MULTIPLE ENDOCRINE CELL NEOPLASIA TYPE 1

The suppressor gene responsible for the MEN-1 syndrome has been located on chromosome 11, band ql3 [25, 26]. Although the gene has not been cloned and sequenced yet, the analysis of numerous available genetic markers tightly linked to the MEN-1 locus with the recent PCR-microsatellite techniques allows identification of allelic loss (loss of heterozygosity, LOH) involving the MEN-1 gene [26]. According to the two hit theory, affected members of the MEN-1 kindreds have inherited the germline genetic defect in the MEN-1 gene whereas the somatic inactivation of the remaining allele by LOH is the causative event for the development of tumors in the involved endocrine glands. Indeed, LOH at 11q13 was found in MEN-1 tumors of pancreatic islets [25], parathyroids [27] and pituitary (for a recent review see [26]).

ECL cell carcinoids associated with the MEN-1 syndrome (type 2) almost exclusively develop in those MEN-1 patients also having ZES. LOH at the MEN-1 gene locus was investigated so far in two of these patients and documented in both [28, 29] (Figure 1). As already mentioned, the genetic defect appears to be crucial for the progression of the
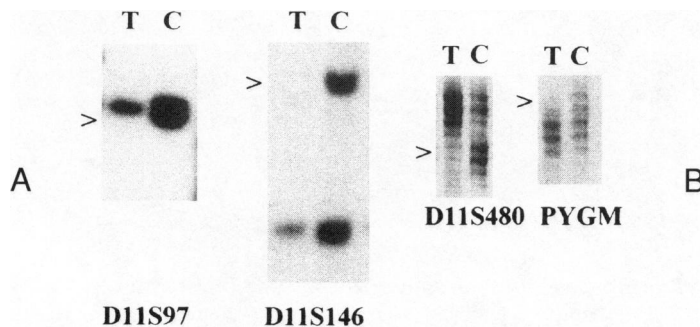

Figure 1. Representative microsatellite-PCR analysis in gastric ECL cell carcinoids (a) and neuroendocrine carcinomas (b) of two MEN-1 patients, with or without ZES, respectively. Analyzed genetic markers tightly linked to the MEN-1 locus are reported below each autoradiogram. Arrows indicate allelic loss in tumor DNA (lane T) in comparison to constitutive DNA (lane C). (Modified from Ref. [29]).

hyperplastic ECL cell proliferations driven by hypergastrinemia to carcinoid tumors. Whether it might have an independent role in tumor induction, however, cannot be ascertained in hypergastrinemic patients with ZES.

Although a sporadic observation of ECL cell hyperplasia in a MEN-1 patient with negligible elevation of serum gastrin was previously reported [6], the clue is likely provided by a recently observed case [29]. This was a female patient with a familial history of MEN-1 but no ZES, no personal history of ZES related symptoms including normal circulating levels of gastrin and absence of gastrinomas or of gastric mucosal hypertrophy at autopsy. Moreover she presented hyperparathyroidism, Cushing's syndrome and multiple islet cell adenomas. At the gastric level multiple tumors immunohistochemically reactive for chromogranin A but unreactive for somatostatin, gastrin, and serotonin were found in oxyntic mucosa of the stomach. They included multiple benign carcinoids (Figure 2a,b) varying in size from 0.5 to 1.5 cm. and two independent, intermediate cell neuroendocrine carcinomas (Figure 2c), which metastatized to gastric lymph nodes and liver. Proliferation of extratumoral oxyntic endocrine cells was not seen except for a mild degree of simple
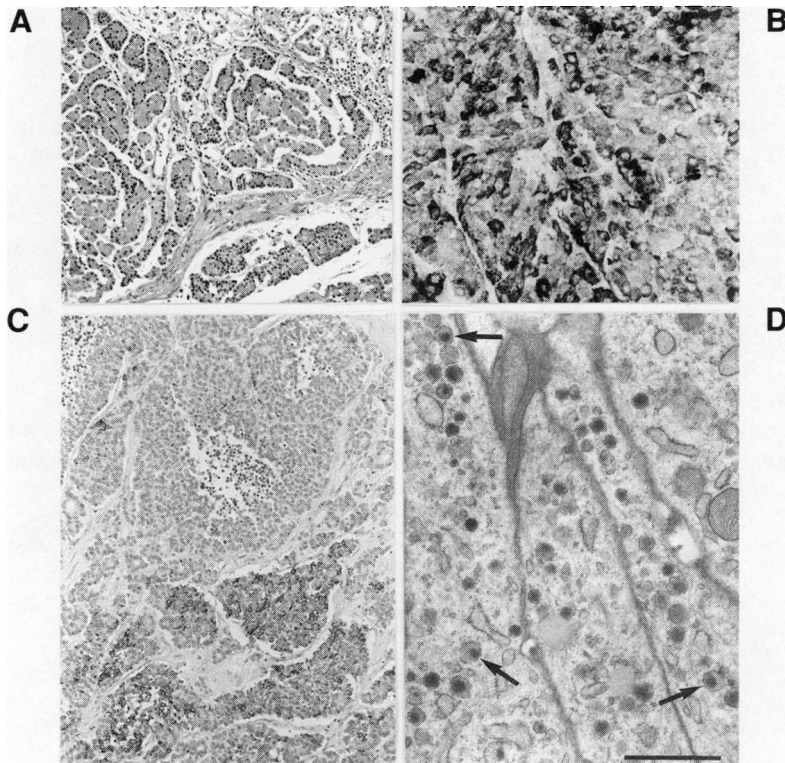

Figure 2. Gastric neuroendocrine tumors in a patient with MEN-1 syndrome but without hyper-gastrinemia. a) One of multiple carcinoids located in the mucosa and submucosa (Hematoxylin-eosin, x 75). **b)** Sevier-Munger argyrophil reaction in the cells of a carcinoid (x 200). **c)** Neuroendocrine carcinoma composed of solid clusters of intermediate size cells with extensive central necrosis. Heterogeneous distribution of chromogranin A immunostaining with abundance of immunoreactive cells in the lower half of the picture (Immunoperoxidase with hematoxylin counterstaining, x 75). **d)** Ultrastructure of the neuroendocrine carcinoma showing secretory granules with small, dense, eccentric cores surrounded by a less dense halo (arrows). Bar is equal to 1 μm. (x 13,600).

hyperplasia. LOH at llql3 region was found in the neuroendocrine carcinomas (Figure 1) that ultrastructurally revealed heterogeneous cell content of granules, characterized by a small, dense and eccentric core, a less dense halo and a wavy limiting membrane (Figure 2d). This granule morphology was consistent with that of modified ECL cells but not with that of any other type of gastric endocrine cells. LOH and ultrastructure could not be investigated in benign carcinoids.

From this observation it can be concluded that multiple gastric endocrine tumors with all evidence composed of ECL cells may develop in MEN-1 patients by LOH at llql3 even in the absence of the promoting stimulus of hypergastrinemia, indicating that the genetic defect per se may be an adequate oncogenic factor for ECL cells.

FEMALE GENDER

A favoring role of the female gender in gastrin-dependent growths of the ECL cells is supported by several lines of evidence. In ZES, either sporadic or associated with MEN-1 syndrome, ECL cell density was found to be higher in women than in men [30, 31]. In addition, women do not present the decrease in ECL cell density with age seen in old men, a finding presented not only by ZES patients [31] but also by normal subjects [32]. Severe hyperplasia of ECL cells of patients with ACG and hypergastrinemia is by far more frequent in females than in males [33]. In this condition, ECL cell carcinoids occur in female patients in two thirds of cases whereas 80% of gastrin independent ECL cell tumors of the sporadic variety (type 3) occur in male patients [1]. These observations are in agreement with the results of experiments in rats with pharmacologically induced hypergastrinemia in which ECL cell carcinoids developed with much higher frequency in female than in male rats [34].

The mechanism that potentiates the trophic effect of gastrin on ECL cells in the female sex is still unclarified. In this regard, it may be relevant the observation of a female patient with ZES and breast cancer, in which an unusually florid evolution of a formerly mild ECL cell hyperplasia abruptly appeared after withdrawal of long-standing anti-estrogen therapy accompanied by increase in circulating levels of 17β-estradiol and progesterone [35].

BCL-2

BCL-2, a protein encoded by the proto-oncogene bcl-2, enhances cell survival by blocking programmed cell death (apoptosis) [36]. Several types of glandular epithelial cells undergoing hyperplasia and apoptotic mediated involution in response to hormonal stimuli express this protein [37]. Activation of the bcl-2 oncogene and related BCL-2 overexpression have been documented in a variety of human tumors originating from different, embryologically unrelated tissues [38, 39]. They contribute to tumor induction and progression by extending cell survival and, therefore, cell exposure to oncogenic factors without affecting the cell proliferation rate.

In our laboratory BCL-2 was found to be consistently expressed by a subset of endocrine cells preferentially located in the intermediate region of the normal human oxyntic glands [40]. This distribution suggests a role in prolonging cell survival during the physiological process of downward migration of these cells. BCL-2 was also commonly expressed in hyperplastic endocrine cells of hypergastrinemic patients that are mostly composed of ECL cells (Figure 3) [6, 24]. Hence, it was argued that the ECL cell is largely involved in such expression and in the related apoptotic regulated mechanism(s).

However, BCL-2 immunoreactivity of ECL cells was found to substantially diverge in hypergastrinemic conditions with different risk of ECL cell carcinoid development (Table 2). In comparison with normal subjects, in fact, the fraction of oxyntic endocrine
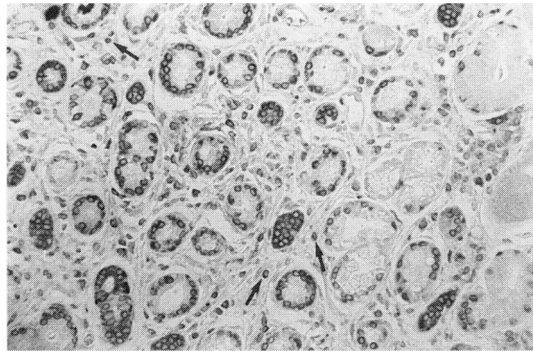
Figure 3. Immunohistochemical expression of BCL-2 by hyperplastic ECL cells in a patient with atrophic gastritis. Stromal lymphocytes also are strongly immunoreactive (arrows). (Immunoperoxidase, x 110).

cells expressing BCL-2 was 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) [40]. In contrast, in established ECL cell carcinoids the expression of BCL-2 appeared to be erratic and in most cases weak or absent even in the presence of strong immunoreactivity of extratumoral, hyperplastic ECL cells. The major role of BCL-2, therefore, seems to be in the early stages of the ECL cell tumorigenic sequence in analogy with similar findings presented by non endocrine gastrointestinal adenocarcinoma [41-43].

Table 2. Ratio of BCL-2 immunoreative cells to chromogranin A (CgA) immunoreactive cells in the oxyntic mucosa of normal subjects and of patients with different conditions associated with hyperplasia of ECL cells (modified from Azzoni et al., 1996).

	Patients n	age	Fasting gastrin (ng/L)	BCL-2/CgA immunoactive cells (percent)
Normal serum gastrin and oxyntic mucosa	10	39 (16-81)	<100	50.0 (24.6-74.0)
Zollinger-Ellison syndrome, sporadic	9	52 (35-61)	1900 (470-29,450)	4.6* (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† (12.1-199.4)

Data are expressed as median (ranges).
*Difference from normal: p<0.001.
†Difference from normal: p<0.006.

The discordant results obtained in different pathological conditions having comparable hypergastrinemic levels indicate that the abnormal regulation of the bcl-2 gene in hyperplastic ECL cells is independent of the influence of gastrin. Indeed, the heaviest degree of BCL-2 expression was found in a very unusual case of severe oxyntic endocrine cell hyperplasia associated with ACG and normal serum levels of gastrin [40]. Nevertheless, our observations may fit with the results of experiments with ^{3}H-thymidine incorporation in pharmacologically induced hypergastrinemia of the rat [44]. These experiments showed that the ECL cell labeling index presented a transient peak elevation during the first two weeks of exposure to hypergastrinemia followed by decline and return to control levels at 10 weeks, in spite of persistent hyperplasia of ECL cells. It was suggested, therefore, that BCL-2 overexpression may represent the mechanism that replaces the early, gastrin dependent peak of mitotic proliferation and extends the life span of formerly proliferated ECL cells [40], thus allowing for the accumulation of genetic and environmental influences necessary for tumor induction.

An interesting, though unexplained finding in both hyperplastic and tumoral ECL cells was the frequent reverse expression of BCL-2 and of the neuroendocrine granule marker chromogranin A [40].

ALPHA SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN (hCG-α)

hCG-α is a protein of unknown functional significance whose expression has been regarded as a marker of malignancy in pancreatic endocrine tumors [45]. It may also be expressed by all other types of endocrine tumors occurring in MEN-1 syndrome [26], although in this case it usually does not carry adverse prognostic significance.

In ECL cells of normogastrinemic subjects hCG-α is usually undetectable. In contrast, it is distinctively and characteristically expressed by ECL cells of hypergastrinemic patients [11, 24, 46]. The volume density of hCG-α immunoreactive cells in the oxyntic mucosa of such patients correlates directly with the degree of their hypergastrinemia (Figure 4a), and the cell content of the protein can be decreased by pharmacological suppression of hypergastrinemia (Table 1) [15, 16]. The more pronounced hCG-α expression is usually observed in ECL cells forming hyperplastic micronodules whereas significantly fewer immunoreactive cells are commonly found in carcinoid tumors [4]. This observation indicates that the gastrin dependent appearance of this novel protein has implications for the induction of ECL cell carcinoids rather than for their progression. When hCG-α is heavily expressed by tumoral ECL cells (Figure 4b), however, it may be associated with unfavorable tumor evolution similar to that found in pancreatic endocrine tumors [29].

BASIC FIBROBLAST GROWTH FACTOR (bFGF)

bFGF controls cell proliferation and differentiation in many organs and tissues, mostly but not exclusively of mesodermal origin. This protein is known to be abnormally elevated in MEN-1 syndrome [47] and largely contributes to the circulating parathyroid mitogen factor that characterizes MEN-1 patients [48].

Using immunohistochemistry and Northern analysis we demonstrated that bFGF is expressed by a subset of unidentified normal oxyntic endocrine cells and by proliferating ECL cells of hypergastrinemic patients including both hyperplastic lesions and carcinoid tumors [49]. 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 a MEN-1 patient showing multiple metastatizing ECL cell carcinoid (Figure 5) [29].
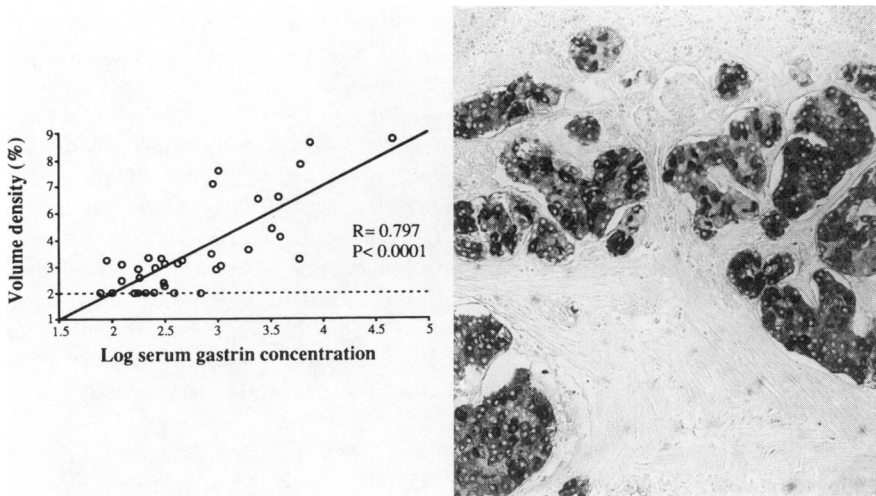
Figure 4. Alpha-subunit of human chorionic gonadotropin (hCG-α) in ECL cells exposed to hypergastrinemia. a) Correlation between plasma gastrin levels and volume density of hCG-α expressing cells [15]. **b)** Unusual pronounced immunoreactivity in an ECL cell carcinoid of a hyper-gastrinemic MEN-1 patient who died of metastatic tumor disease in the liver (Immunoperoxidase without counterstaining, x 130).

Whether bFGF production by ECL cells may have some autocrine influences on the induction of ECL cell carcinoids remains to be elucidated. It has been suggested that locally released bFGF, a potent mitogen for smooth muscle cells, may represent the growth factor responsible for the pronounced stromal proliferation of smooth muscle cells originating from the muscularis mucosae that is frequently associated with ECL cell micronodules or carcinoid tumors [50].

Figure 5. Heavy immunoreactivity for basic fibroblast growth factor in hyperplastic ECL cells of a MEN-1 patient (arrows). Carcinoid tumor cells (on the right) display a lower degree of immunostaining. (Immunoperoxidase, x 70).
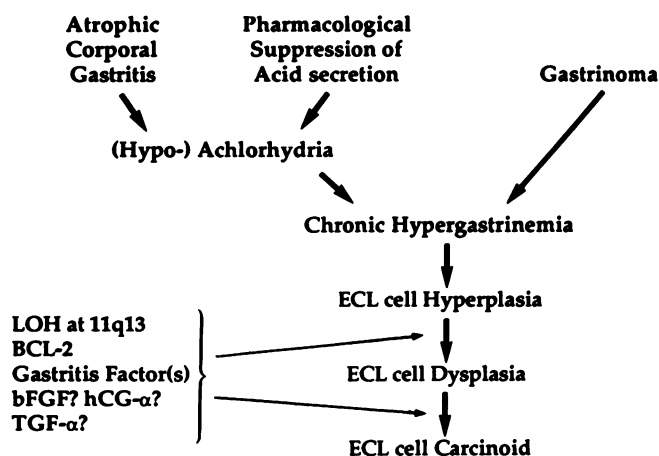
Figure 6. Outline of the mechanism involved in the induction of gastrin-dependent ECL cell carcinoids.

INSIGHTS FROM EXPERIMENTAL MODELS

Several experimental models of ECL cell tumors have been proposed (for a recent review see [14]). Of them the ECL-omas of the rodent *Mastomys* appear to be the most promising one since it may reflect either the genetic mechanism, when spontaneously developing in normogastrinemic old animals, or the gastrin dependent mechanism in animals made hypergastrinemic by pharmacological treatment with the irreversible H_2 blocker loxtidine that results in a significantly accelerated tumor formation [14]. During the hypergastrinemia driven induction of *Mastomys* ECL-omas, increased ECL cell content of the mitogenic agent transforming growth factor-α (TGF-α) and of its specific EGF receptor have been documented. Accordingly, the hypothesis has been formulated that gastrin-dependent TGF-α production and related autocrine stimulation may be responsible for a gastrin-independent phase of ECL cell proliferation [14]. TGF-α production by human ECL cells has not been documented so far.

CONCLUSIONS

The clinical and pathological evidence indicates that in hypergastrinemic patients gastrin is the dominant agent responsible for ECL cell proliferation at all steps of the sequence hyperplasia-dysplasia-neoplasia. Hypergastrinemia appears to behave as a promoter but apparently lacks transforming properties as shown by the absence of ECL cell carcinoids in patients exposed to hypergastrinemia alone, i.e. those with sporadic ZES. On the basis of the available knowledge the potential transforming factors responsible for the development of gastrin-dependent ECL cell carcinoids (type 1 and 2) may be subdivided into: 1) factor with proven oncogenic activity such as the allelic loss of the MEN-1 suppressor gene in the genetically predisposed MEN-1 patients, an alteration that may induce ECL cell tumors even in the absence of hypergastrinemia; 2) factor(s) still unknown such as those associated with ACG; 3) agents whose role in the induction of human ECL cell tumors is still unclarified, such as bFGF, hCG-α and TGF-α; and 4) agents having a favoring role on the ECL exposure to mitogens such as BCL-2. A schematical outline of the process of ECL cell tumorigenesis in hypergastrinemic states is shown in Figure 6.

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