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Relationship of ECL Cells and Gastric Neoplasia

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The enterochromaffin-like (ECL) cell in the oxyntic mucosa has a key role in the regulation of gastric secretion since it synthesizes and releases the histamine regulating the acid secretion from the parietal cell. Gastrin is the main regulator of the ECL cell function and growth. Long-term hypergastrinemia induces ECL cell hyperplasia, and if continued, neoplasia. ECL cell carcinoids occur in man after long-term hypergastrinemia in conditions like pernicious anemia and gastrinoma. There is also accumulating evidence that a proportion of gastric carcinomas of the diffuse type is derived from the ECL cell. Furthermore, the ECL cell may, by producing substances with angiogenic effects (histamine and basic fibroblast growth factor), be particularly prone to develop malignant tumors. Although the general opinion is that gastrin itself has a direct effect on the oxyntic mucosal stem cell, it cannot be excluded that the general trophic effect of gastrin on the oxyntic mucosa is mediated by histamine or other substances from the ECL cell, and that the ECL cell, therefore, could play a role also in the tumorigenesis/carcinogenesis of gastric carcinomas of intestinal type.

INTRODUCTION

The enterochromaffin-like (ECL)[b] cell was identified in the oxyntic mucosa in the sixties [1] and accepted as the cell producing and storing the histamine taking part in the regulation of gastric acid secretion in the rat [2]. Subsequently the ECL cell was described in other species including man, but histamine was not found in the ECL cells in non-murine species until the latter part of the eighties [3]. It was then shown that the stimulatory effect of gastrin on acid secretion could be solely explained by stimulation of histamine release from the ECL cell [4]. Histamine is released from the ECL cells at the base of the glands to the capillaries, thereby reaching all the parietal cells in the same gland [4, 5]. Gastrin was soon recognized as an important stimulant of the ECL cell growth inducing not only ECL cell hyperplasia, but also ECL-omas in the rat [6]. Moreover, the function of the ECL cell (synthesis and release of histamine) showed the same concentration response dependence to gastrin as did ECL cell proliferation [7], suggesting interaction with the same receptor. However, based upon cloning of the gastrin receptor from purified parietal cells, the gastrin receptor was claimed to be situated on the parietal cell [8]. The presence of a gastrin receptor on the parietal cell has not been confirmed in functional studies, whereas a gastrin receptor has been demonstrated functionally on ECL cells [9] where gastrin receptor mRNA expression also has been demonstrated [10]. It is, therefore, indisputable that there is a gastrin receptor regulating the function as well as growth of the ECL cell, whereas it is doubtful whether there is such a receptor on the parietal cell.

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[b]*Abbreviations:* ECL, enterochromaffin-like; CgA, chromogranin A; HDC, histidine decarboxylase; bFGF, basic fibroblast growth factor; TGFα, transforming growth factor α; H_2, histamine$_2$; H_1, histamine$_1$; MEN, multiple endocrine neoplasia.

ECL CELL FUNCTION

Gastrin stimulates the release of histamine from the ECL cell and at the same time the synthesis of histamine at least partially by increasing histidine decarboxylase (HDC) mRNA abundance [11]. Histamine release has been reported to be stimulated by cholinergic agents [12], although the histamine releasing activity of these agents is at best marginal [13].

The ECL cell furthermore stores chromogranin A (CgA), and gastrin seems to regulate the release of the CgA split product pancreastatin and of histamine in the same way [14]. Calbindin [15] basic fibroblast growth factor (bFGF) [16], glycoprotein hormones [17], and transforming growth factor α (TGFα) [18] have also been reported to be stored in the ECL cell. The regulation of the release of these substances has not been clarified.

ECL CELL GROWTH REGULATION

Gastrin is the main regulator of ECL cell proliferation. Thus, hypergastrinemia induces ECL cell hyperplasia [6], while hypogastrinemia reduces ECL cell proliferation [19]. Accordingly, gastrin in physiological concentrations does affect ECL cell proliferation and ECL cell density. However, prolonged hypergastrinemia inducing marked ECL cell hyperplasia results in a normalization or even reduction of the ECL cell proliferation [20]. The apparent reduction in gastrin-stimulated ECL cell proliferation rate persists as long as ECL cell hyperplasia lasts [20]. This suggests that the ECL cell itself produces a factor inhibiting its own proliferation. Because the histamine$_2$ (H_2) antagonist loxtidine seems to induce ECL cell carcinoids in more species than the proton pump inhibitor omeprazole [21], we explored the effect of histamine on ECL cell proliferation. We found that the histamine$_1$ (H_1) antagonist astemizole tended to have a slight stimulatory effect on ECL cell function and ECL cell proliferation [22], and the unsurmountable H_2 receptor antagonist loxtidine gave a slightly more pronounced increase in the density of argyrophilic cells compared with omeprazole in spite of lower gastrin levels [21]. The effect of histamine on ECL cell proliferation has also been studied by Modlin and co-workers [23]. They found that the H_1 antagonist cyproheptadine reduced ECL cell hyperplasia induced by hypergastrinemia [23]. It can be concluded that the role of histamine as a regulator (inhibitor) of ECL cell growth is not yet settled. However, the fact that the ECL cell proliferation is reduced as long as ECL cell hyperplasia persists [20] indicates that the ECL cells produce a factor reducing its own proliferation.

TUMORIGENIC FACTORS FROM THE ECL CELL

Vascular effects

Histamine has an angiogenic effect [24], which could be of importance in tumorigenesis. Moreover, the effect of histamine on capillary permeability would promote passage of cells from the tissue into the blood stream and, thus, facilitate metastasis. bFGF produced in the ECL cell [16] has also an angiogenic effect [24]. Thus, substances normally produced in the ECL cell facilitate tumor growth and metastases. This could be a general phenomenon of neuroendocrine cells explaining the apparent discrepancy between the low degree of cellular atypia and the clinically malignant behavior of such tumors. In other words, these cells may be more likely to develop tumors, including malignant ones, due to their normal production of paracrine factors. Therefore, tumors may arise from such cells after fewer mutations and with less deviation from the normal cell than tumors from cells normally lacking the production of such tumorigenic factors.

Effects of ECL cell products on the proliferation of other cells in gastric mucosa

Whereas it is now accepted that the stimulation of acid secretion by gastrin is mediated by the stimulation of histamine release [4], gastrin stimulatory effect on stem cell proliferation in the oxyntic mucosa [25] is thought to be a direct effect of gastrin. However, the presence of a gastrin receptor on the parietal cell [8] has not been confirmed, and it could also be that the gastrin stimulatory effect on stem cell proliferation is a secondary one (Figure 1). We have previously suggested that histamine could mediate also this effect of gastrin [26], and we have given experimental arguments in favor of this view [21]. We could not, however, find that the H_2 agonist impromidine increased the stem cell proliferation [27]. Anderson and co-workers furthermore showed that the general trophic effect of gastrin in the oxyntic mucosa was apparently not dependent on histamine since hypergastrinemia in combination with the histidine decarboxylase inhibitor α-fluoromethyl histidine induced an increase in the oxyntic mucosal thickness [28]. Later we could show that although α-fluoromethyl histidine does reduce gastrin stimulated histamine release, it does not abolish it [14]. Therefore, the general trophic effect of gastrin on the oxyntic mucosa can nevertheless be mediated by histamine. As stated above, the ECL cell produces many different factors, and it is possible that some of these like bFGF [29] or histamine [26, 30] could affect the proliferation of the stem cell. The localization of the ECL cell at the base of the gastric glands makes it possible for substances to reach not only the parietal cells, but also the stem cells, via the capillaries. It may, therefore, be concluded that the ECL cell could play an important role not only in the regulation of the function, but also the proliferation of the cells in the oxyntic mucosa.

TUMORS THAT ARISE OR MAY ARISE FROM THE ECL CELL

It is accepted that murine neuroendocrine cells do proliferate, whereas it has been stated that human gastrointestinal neuroendocrine cells do not [31]. The latter conclusion

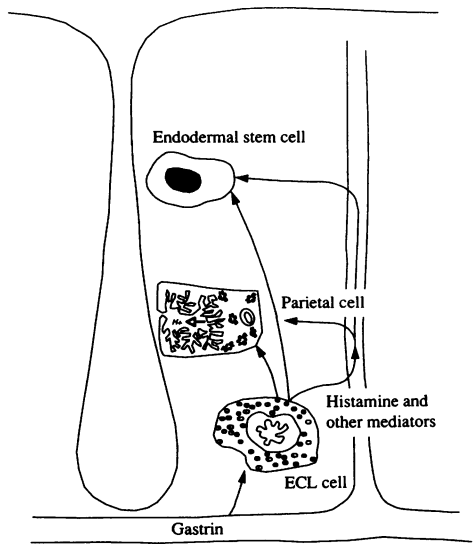

Figure 1. Products from the ECL cell can reach and affect the function of the parietal cell as well as the proliferation of the endodermal stem cell by substances released to the capillaries.

was obviously based on an insufficient number of cells studied [32], and taking into consideration that gastrin induces hyperplasia and ECL-omas in man [33] as well as murine species [6], it seems clear that also the human ECL cell does proliferate. The ECL cell, being the most predominant neuroendocrine cell in the oxyntic mucosa [34], is the most common origin of carcinoids arising in that tissue [35]. Moreover, hypergastrinemia predisposes to the development of carcinoids in the oxyntic mucosa and more specifically to ECL-omas whether there is anacidity/hypoacidity [36] or increased acid secretion [33]. It has been claimed that hypergastrinemia alone is not sufficient to induce ECL-omas, and that ECL-omas in patients with gastrinomas occur exclusively in patients with concomitant multiple endocrine neoplasia (MEN) [33]. However, spontaneous gastrinoma may also be accompanied by ECL-omas [35, 37], and ciprofibrate, a stimulator of peroxisome proliferation, induces ECL-omas [38] without inhibiting gastric acid secretion [39]. Moreover, ciprofibrate influences the function of the D-cell in the antrum where it is of the open type, but not in the oxyntic mucosa [40], suggesting that the ECL cells in ciprofibrate treated rats are influenced by hypergastrinemia alone. Therefore, hypergastrinemia seems to be sufficient to induce ECL-omas, and it is presently impossible to decide whether gastritis in the oxyntic mucosa plays a separate role in ECL cell tumorigenesis [41]. This is, indeed, an important question since *Helicobacter pylori* (*HP*) infection induces hypergastrinemia [42] and also predisposes to gastric malignancies [43]. ECL-omas developing in patients with hypergastrinemia are more benign than those developing without hypergastrinemia (so-called spontaneous ECL-omas) [35]. This is hardly surprising, since hypergastrinemia will be expected to stimulate the proliferation not only of normal ECL cells but also slightly modified ECL cells with a few mutations. ECL-omas occurring without hypergastrinemia most probably have greater derangements in the growth control indicating more mutations or mutations with more severe functional effects. There is no reason to doubt that ECL-omas developing in normogastrinemic individuals are on an average more malignant than those found in hypergastrinemic individuals [35], although some of the latter ones also show malignant behavior both in the rat [44] and man [35]. ECL cell hyperplasia secondary to hypergastrinemia in the rat [45] and in man [46] has been reported to be reversible. It is, however, highly unlikely that all mutated cells will disappear when normogastrinemia is restored. It is more likely that abolishing hypergastrinemia will only reduce the number of mutated as well as normal ECL cells. This view is also supported by a study on *Mastomys* showing that ECL-omas developed in *Mastomys* treated for 24 weeks with an inhibitor of gastric acid secretion and then observed for 24 more weeks without any treatment [47]. Therefore, hypergastrinemia obviously induces mutations in ECL cells since it alone leads to tumors including malignant ones from that cell. Accordingly, even short-term hypergastrinemia may give a long-term increased risk of ECL cell derived tumors.

As for other carcinoids, the fraction of tumor cells in ECL-omas demonstrating markers specific for ECL cells or even neuroendocrine markers varies [35]. Rindi and co-workers, in their retrospective study on 55 gastric tumors with previously established neuroendocrine nature, described 35 cases as related to hypergastrinemia and being relatively benign although two of them metastasized. There were ten so-called sporadic ECL-omas, most of them metastatic or locally invasive and, furthermore, nine so-called neuroendocrine carcinomas showing high mortality [35]. Whereas the ECL cell was accepted as the cell of origin in the carcinoids, the neuroendocrine carcinomas (the distinction between metastasizing carcinoids and neuroendocrine carcinomas, was not clearly given) were claimed to be of proto endocrine nature and of possible endodermal origin [35]. However, although the secretory granules in these malignant cells were impossible to classify due to their atypical appearance, some tumor cells were Grimelius positive (range five to 80 percent) and serotonin negative [35]. Staining these tumors by the Sevier

Munger method [48] may have classified also these tumors as ECL cell-derived [49]. Interestingly, they described a gland-like growth pattern both in the spontaneous ECL-omas and the so-called neuroendocrine carcinomas [35]. It is, indeed, difficult to distinguish between neuroendocrine tumors and adenocarcinomas by light microscopy alone, as demonstrated in retrospective studies on human tumors [50], the reclassification of the tumors developing in rats during life-long hypergastrinemia [44, 51], and the gastric tumors of *Mastomys* [52, 53]. While Rindi et al. studied previously accepted neuroendocrine gastric tumors [35], we have re-examined gastric tumors previously classified as adenocarcinomas [54]. We found that seven of 19 (36 percent) of tumors of diffuse type and none of ten tumors of intestinal type (classified according to Laurén [55]) contained Sevier Munger positive and diazo and serotonin negative tumor cells [54,56] compatible with an ECL cell origin [49]. The fraction of tumor cells having staining qualities compatible with an ECL cell origin varied from less 0.5 percent to 10 percent [54]. However, as there is a continuous reduction in the percentage of recognizable ECL cells in the more typical ECL-omas [35], it is hardly logical to dismiss the ECL cell origin of tumors even when only a few tumor cells show specific staining. In this context it should be recalled that the diagnosis of adenocarcinoma is made upon glandular growth pattern and mucine positivity. However, mucine positivity reflects only the presence of glycoproteins, including glycoprotein hormones [57] which are found in ECL cells [17]. Mucine positivity can, therefore, hardly be regarded as a specific staining for exocrine cells. Furthermore, as previously discussed, glandular growth pattern may also be found in neuroendocrine tumors [35, 58]. Therefore it may be concluded that the ECL cell could be the cell of origin not only for most of the carcinoids localized to the oxyntic mucosa, but also for a proportion of the tumors presently classified as adenocarcinomas. This may particularly be the case for adenocarcinomas of the diffuse type. Although most patients with gastric carcinoma do not have hypergastrinemia at the time of diagnosis, it cannot be excluded that previous hypergastrinemia of shorter or longer duration could have contributed to the tumorigenesis. Moreover, gastrin receptors have been described on gastric carcinoma cell lines, and interestingly only on cell lines derived from carcinomas of the diffuse type [59, 60].

If hypergastrinemia at one stage or the other plays an important role in the tumorigenesis leading to gastric carcinomas of the diffuse type, such patients would be expected to have higher gastrin values than those with tumors of the intestinal type [61]. This is, however, not the case [62] although there is a report describing higher gastrin values in patients with diffuse type of gastric carcinoma [63]. However, the ECL cell is the only among seven neuroendocrine cells in the oxyntic mucosa [64] where gastrin has a positive trophic effect, whereas gastrin has a negative trophic effect on the oxyntic D-cell [65]. Thus, the D-cell could play a role in the tumorigenesis in conditions with long-term hypogastrinemia such as after gastric resection [66]. Therefore, due to the heterogeneity of the neuroendocrine cells, and the differences in the effect of gastrin on these cells with regard to their proliferation, attempts to correlate gastrin in blood and the occurrence of diffuse gastric carcinomas may not disclose important relationships in subgroups of the patients. Moreover, a correlation between type of gastric carcinoma and gastrin in blood should only be assessed when studying tumors arising in the oxyntic mucosa since the ECL cell is exclusively found in this area.

It should be realized that we [54] and others [50, 67] have found that neuroendocrine carcinomas have been underestimated in the classification of gastric carcinomas. Staren and co-workers reclassified both tumors of poorly differentiated and moderately differentiated adenocarcinomas to neuroendocrine carcinomas based upon histochemical staining qualities of tumor cells [67]. Although neuroendocrine gastric carcinomas previously most often have been misclassified as being gastric carcinomas of the diffuse type, they can, [67] like carcinoids [58], show a glandular growth pattern and, therefore, be classified as carcinomas

of intestinal type. Nevertheless, neuroendocrine differentiation in tumor cells is found much more often in gastric carcinomas of the diffuse type compared with the intestinal type [68]. Chejfec and Gould suggested as early as in 1977 that some undifferentiated gastric carcinomas were derived from neuroendocrine cells [50], and in 1956 Waldenstrøm and co-workers described a patient with a malignant tumor releasing histamine secondary to food intake, thus probably representing a malignant ECL-oma [69]. There are many observations suggesting a fundamental biological difference between gastric carcinomas of diffuse and intestinal type. Thus, tumors do not change from one type into the other in the individual patient [55]; in epidemiological studies, intestinal but not diffuse gastric carcinomas show a reduced incidence [70], and there is a difference in sensitivity towards cytotoxic drugs between the two types of gastric carcinoma [71]. Interestingly, bFGF is detected and its mRNA is expressed more often in gastric tumors of undifferentiated type compared with differentiated ones, particularly in the scirrhous variant [72]. It should be recalled that bFGF is produced in the normal ECL cell [16], and it might be that the well known fibrosis in these carcinomas [73,74] is due to a factor like bFGF being released from a neuroendocrine tumor cell [75]. Fibrosis, locally [76] or remote from the tumor (like the valves of the heart) [77], is a well known accompanying reaction to midgut carcinoids. Some diffuse carcinomas also induce a hyperplasia of the covering gastric mucosa [78]. These carcinomas often show endocrine differentiation, and interestingly most of them occur in young women [78]. In that context it should be recalled that ECL cell carcinoids develop more frequently in female than in male rats [6, 44]. Ooi and co-workers, however, described no proliferative activity of tumor cells with neuroendocrine differentiation in gastric carcinomas [79]. An alternative interpretation of their finding is that the most rapidly dividing tumor cells are the most dedifferentiated ones and, thus, the cells without specific markers [80]. Therefore, there are many indications suggesting that at least a part of the gastric carcinomas of diffuse type actually are neuroendocrine tumors (Figure 2).

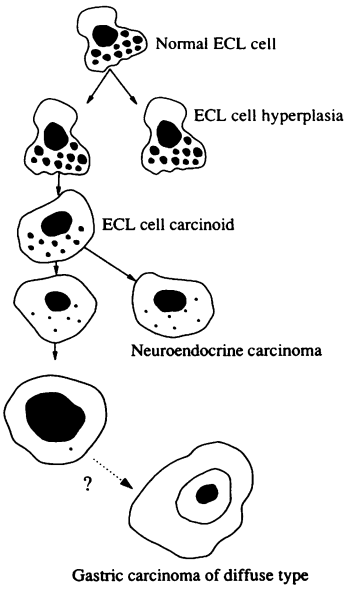

Figure 2. The ECL cell may give rise to gastric carcinoids, neuroendocrine carcinomas and possibly gastric carcinomas (mainly of the diffuse type).

Gastric carcinoma of intestinal type

Since the ECL cell is localized to the oxyntic mucosa only, this cell probably does not influence the growth and, thereby, tumorigenesis in other parts of the stomach. Nevertheless, long-term treatment of mice with potent and irreversible H_2-antagonists has been reported to induce not only tumors in the oxyntic mucosa but also in the forestomach [81]. The pathogenesis of these tumors in the forestomach was not disclosed, but it cannot be excluded that they could be due to stimulation of the proliferation of the stem cell in the border zone between the squamous epithelium and the oxyntic mucosa secondary to substances released from an increased ECL cell mass. The release from these ECL cells could be particularly exaggerated not only due to hypergastrinemia secondary to an inhibition of acid secretion, but possibly also by an attenuation of the histamine restraint on ECL cell release due to the H_2-receptor blocking effect of the drug [82]. If gastrin mediates the general trophic effect on the oxyntic mucosa by stimulating the release of histamine and other substances with mitogenic activity (see above), it is not unlikely that gastrin could play a role in the tumorigenesis of gastric carcinomas of intestinal type as well. There are, in fact, observations suggesting that products from ECL cells can induce growth derangements in nearby mucosa. Thus, the mucosa covering ECL-omas in *Mastomys* may show dysplasia. The pathogenesis of hyperplastic and adenomatous polyps developing in hypergastrinemic patients with atrophic gastritis type A [83] is not known, but hyperplastic polyps have been described together with gastric carcinoma of the diffuse type [84], and also recently in patients on long-term omeprazole treatment (G. Brunner, Hannover, Germany. Personal communication). So-called collision tumors with one part of the tumor being a typical carcinoid while the other is a differentiated adenocarcinoma have also been described in the oxyntic mucosa [85]. In such cases, the adenocarcinoma could be induced by the release of paracrine mediators from the carcinoids. On the other hand, the so-called composite gastric carcinoids and adenocarcinomas where the tumors show a mixture of carcinoid and carcinomatous tissue at different places, the so-called adenocarcinoma part, has as a rule been of the diffuse type [86]. In such cases the carcinoma component may only represent a further malignization of the carcinoid neuroendocrine tumor cell.

CONCLUSION

The ECL cell has a central role in the regulation of the function of the oxyntic mucosa and may have a similarly central role in the regulation of mucosal growth in the same area. There is no reason to doubt that the human ECL cell, like its murine counterparts, does have the ability to self-replication. Gastrin is the main regulator of ECL cell function and growth. Hypergastrinemia induces ECL cell hyperplasia and ECL cell carcinoids, and since tumorigenesis is a continuous process, this will necessarily predispose to the development of more malignant ECL derived tumors like ECL cell neuroendocrine carcinomas and probably some of the tumors presently classified as gastric carcinomas of diffuse type. ECL cell-derived tumors developing in normogastrinemic individuals tend to be more malignant than those developing in patients with hypergastrinemia, probably reflecting gastrin independent growth in these so-called spontaneous ECL-omas. By release of its different mediators, the ECL cell could also contribute to the tumorigenesis from the endodermal stem cell leading to carcinomas of intestinal type. Iatrogenic hypergastrinemia should be avoided in young individuals since it would be expected to increase the frequency of gastric carcinomas [87].

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