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ENTEROCHROMAFFIN-LIKE CELLS AND GASTRIC ARGYROPHIL CARCINOIDOSIS

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Abstract

The relevance of enterochromaffin-like (ECL) cells in gastric pathobiology has generated considerable interest particularly since the recent description of a pathological state characterized as gastric argyrophilic carcinoidosis. The morphological and biofunctional properties of these cells are distinct from other gastric endocrine cells. It is probable that ECL cells have a major role in the regulation of parietal cell function. Other possible functions may include a trophic regulatory influence. Of particular interest is the recent observation that agents which result in profound and sustained acid inhibition may cause ECL cell hyperplasia. This phenomenon has also been noted in human disease states in which a significant decrease in acid section is evident (pernicious anemia/atrophic gastritis). In patients with gastrinomas of the multiple endocrine neoplasia type I group, therapeutically induced acid inhibition may result in gastric ECL hyperplasia and even neoplasia (gastric carcinoid or ECLoma). Similarly, in the rodent species mastomys, which is genetically predisposed to the formation of gastric carcinoids, exposure to acid inhibitory agents results in rapid (90–120 days) development of gastric carcinoids. The pathobiological relevance of ECL cells and the mechanisms of their inducible hyperplasia and neoplasia may be of considerable significance in understanding the regulatory role of gastric endocrine cells.

Key words: Enterochromaffin-like cell, gastric carcinoid, mastomys, argyrophilic carcinoidosis.

A large number of different neuroendocrine cells have been identified in the human gastrointestinal tract. Their distribution ranges from the distal esophagus to the rectum. Certain cell types have been identified throughout the gastrointestinal tract while others are localized to specific organs or sites within these organs (1). A number of these cells contain colocalized peptides and amines which presumably function as gastrointestinal regulatory peptides. Despite a substantial array of different secretory products, these cells share certain common immunocytochemical and ultrastructural characteristics. Overall it seems apparent that they function in the modulation of various aspects of gastrointestinal physiology via endocrine, neurocrine, and paracrine interactions.

Gastrointestinal endocrine cell classification

At least seven different endocrine cell types have been identified in the human stomach. These include the EC, D, P/D1, A (fetus and newborn), X, G, and ECL cells (1–3). Gastrin secreting cells are limited to the antral mucosa while the X and ECL cells are confined to the oxyntic mucosa. The other cell types are evident in both the oxyntic and antral mucosa. Endocrine cells represent approximately 2% and 1.2% of the normal oxyntic mucosa in the rat and human respectively (2, 4). The most frequent neuroendocrine cell type present in the oxyntic mucosa of the rat and human is the enterochromaffin-like (ECL) cell. In the rat they comprise at least 65% of the fundic argyrophilic cell population, while in the human the percentage appears to be slightly lower (40–60%) (5). The exact function and precise peptide/amine content of human ECL cells is to a large extent undetermined. However, their involvement in several pathophysiological states is apparent. These include atrophic gastritis (6), pernicious anemia (7, 8), Zollinger-Ellison syndrome (4, 9), and gastric argyrophilic carcinoidosis (10). Current investigation has focused considerable attention on the putative role of ECL cells in gastric physiology as well as in these disorders.

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The affinity of neuroendocrine cells of the gastrointestinal tract for various silver stains has long been recognized. In 1966, Håkanson et al. (11) identified a system of cells in the rat oxyntic mucosa that, like enterochromaffin cells, was capable of amine precursor uptake and decarboxylation. The following year they reported that these cells were identical to histamine-containing cells of the oxyntic mucosa and introduced the term 'enterochromaffin-like cells' (12, 13). Capella et al. (14) in 1969 reserved the term 'enterochromaffin-like' for a distinct oxyntic endocrine cell type identified by the characteristic electron microscopic appearance of its individual secretory granules. Subsequent identification and classification of the gastric neuroendocrine cells has been based largely on their different staining characteristics and the ultrastructural morphology of their secretory granules as determined by electron microscopy (15, 16). In addition, identifiable storage and secretory products provide additional information useful in further classification and identification (17).

At the light microscopic level, human ECL cells are small, irregularly shaped cells with pale cytoplasm, and nuclei which usually exhibit fine nuclear membranes and chromatin (18). Cytoplasmic granules are either not evident by light microscopy or only evident as fine indiscrete granules (18). They are argyrophilic and stain positively with Grimelius and Sevier-Munger techniques. They are negative with Hellerström-Hellman and Masson techniques (15). In many species, unprepared ECL cells, though not argentaffin staining, may become so after treatment with amine precursors (1). Ultrastructural studies have suggested that certain ECL cells of the cat and rabbit may display argentaffin granules even without pretreatment (1). Some human ECL cell granules may also show argentaffin staining but can be differentiated from EC cells by other histochemical staining techniques such as the lead-hematoxylin and hydrochloric acid-base dye techniques (1).

In the gastric fundic mucosa, ECL cells are located in the deep portions of gastric pits. Few ECL cells have been identified in the neck of the glands and no ECL cells are present among the gastric surface cells. Similar to other endocrine cells of the gastric fundic mucosa, ECL cells lie deep to the oxyntic or peptic cells and possess no appreciable contacts with the lumen of the glands (1).

The secretory products of several of the gastric neuroendocrine cells are well characterized and can therefore be utilized in immunocytochemical identification and localization of these cells (e.g. gastrin in G cells and serotonin in EC cells). However, the secretory product(s) of ECL cells (aside from histamine which has been demonstrated in many species (18)) remain unknown or open to conjecture. Identification of ECL cells by immunocytochemical staining is therefore limited either to antigens such as histamine (which is also found in the oxyntic mucosal mast cells) and histidine decarboxylase (19–21) (which is also found in EC and mast cells), or antigens common to many other neuroendocrine cells. These include neuron-specific enolase (22) and chromagranin (23). Precise identification of ECL cells may, therefore, require immunohistochemical staining procedures in addition to electron microscopic ultrastructural studies (24). Multiple staining and subtraction techniques may then be necessary to identify and quantify ECL cells at the light microscopic level (25).

At the ultrastructural level, human ECL cells exhibit many features in common with other gastric neuroendocrine cells and ECL cells of other species. These primarily include subcellular organelles typical of secretory cells. Nuclei are large, prominent, and occupy a substantial portion of the cell, while granules are evident predominantly in the basal cytoplasm (18). Desmosomes with neighboring plasma membranes are occasionally identifiable. Typically, granules are of two types. Either large and vesicular with an irregularly dense core located eccentrically: or small, compact, and nonvacuolated with a coarse core separated from a wavy membrane by a thin space (1, 2, 18). Most cells predominantly display the small round granules or both types. Only rare cells exhibit the vacuolated granule alone (18). ECL cells also contain numerous small, dense mitochondria, prominent filaments, and much of their endoplasmic reticulum is smooth (19).

ECL cell ontogeny

The origin of gastric neuroendocrine cells in general and ECL cells in particular remains unresolved. Although both endodermal and neuroectodermal origins have been proposed and studied, the concensus remains that the endodermal theory may be the better documented (26–28). While in depth, studies of the appearence and distribution of ECL cells during development are relatively few in number, it is apparent that ECL cells are scarce in the human fetus (1). ECL cells in the fundic mucosa of the human fetus have been observed ultrastructurally and/or immunocytochemically at approximately the fourteenth week of development (29).

A recent report of ECL cell populations in normal adult males and females noted that ECL densities declined with age in males as opposed to females (25). Significant, however, is the fact that circulating gastrin levels were found to be higher in older females than in younger or older males, and to be associated with a higher incidence of gastritis (25). It has been well documented that ECL densities are influenced by variations in circulating gastrin levels (vide infra).

The question of ECL cell replication has been a source of some conjecture. Tielemans & Willems (2) addressed ECL cell renewal in mouse oxyntic mucosa using ^{3}H-thymidine kinetic studies. They reported slow self-replication of ECL cells through mitosis without evidence of differentiation from stem cells. ECL cell cycle time was estimated at about two months in this study. Rat ECL cell

proliferation was similarly studied in rats made hypergastinemic by either omeprazole treatment or antral exclusion. Under these conditions, it was concluded that ECL cell hyperplasia occurred by self-replication (2, 30). While it is presumed that similar mechanisms of ECL cell renewal and hyperplasia are present in humans, appropriate studies are currently not available.

ECL cell secretion

Although ECL cells have been well characterized at the ultrastructural level, little is known about either the contents of their secretory granules or the regulation of their secretion. A number of studies have suggested that ECL cells are variously under the control of either humoral or neuronal stimuli (15, 31–35). Evidence for the presence of gastrin and acetylcholine receptors on ECL cells in several species, although not in humans, exists (31). In rat ECL cells, gastrin has been reported to result in histamine release and activation of histidine decarboxylase (15, 32–34). This is associated with a reduction in the number of granules per unit of cytoplasm in ECL cells (15, 34, 35). Prolonged stimulation by gastrin is associated with enlargement of the Golgi complex and endoplasmic reticulum and an increase in cell size (20, 34–37). Hyperplasia of human ECL cells during states of hypergastrinemia resulting from pernicious anemia (6, 7), atrophic gastritis (5), and Zollinger-Ellison syndrome (3, 38) has been well documented. Similarly, ECL cell hyperplasia has been experimentally produced in several species by rendering them hypergastrinemic either by antral exclusion (35), or the exogenous administration of pentagastrin (32). Conversely, procedures which decrease gastrin levels, such as antrectomy, have shown a decrease in ECL cell numbers from baseline values (32). ECL hyperplasia, sometimes progressing to gastric argyrophil carcinoidosis, has been demonstrated in several species in studies of long-term acid secretory suppression with H_2-histamine receptor antagonists and omeprazole (39–41). Although the secretory effects of gastrin may be suppressed by these compounds, the trophic effects appear unaffected (2, 42). This may be interpreted as blockade of secretion alone while trophic action is unapposed. Alternatively, it raises the possibility that a second regulator (cellular or biochemical) of trophic activity may exist. When taken together these studies would appear to support a central role for gastrin in the control of ECL kinetics and function.

Håkanson et al. (34, 43) have reported that in the rat unilateral subdiaphragmatic vagal denervation is accompanied by a pronounced reduction in the weight and height of the oxyntic mucosa. In addition, a parallel decrease in the number of endocrine cells in the oxyntic area of the denervated side was noted. The neuronal pathway and transmitters involved are unknown. Conversely, bilateral vagal denervation failed to result in a similar reduction in mucosal height and weight or endocrine cell number. The lack of effect was interpreted to be due to a positive trophic effect of the relative hypergastrinemia consequent upon increased antral pH following bilateral vagotomy.

Portocaval shunting has been reported to result in an increase in the ECL cell population of the rat (32, 34, 44). This effect is independent of gastrin since it occurs in the absence of hypergastrinemia. Furthermore, shunting has no trophic effect on the remainder of the oxyntic mucosa (34, 44). It has been proposed that the substance responsible for this ECL cell trophic effect may be a circulating factor whose biological activity is accentuated consequent upon an impaired degradative capacity of the liver secondary to shunting (15, 34). Thus, both unilateral vagal denervation and portocaval shunting studies in the rat have demonstrated trophic effects of the ECL cell population in the absence of elevated gastrin levels. It is thus probable that other factors influence ECL cell function.

ECL cell pathophysiology

The relevance of the ECL cell to gastric pathobiology has in more recent times become a subject of considerable interest. Its exact and ultimate pathobiological significance is, however, as yet not clear. Gastric carcinoids represent approximately 3–4% of all carcinoids (45, 46) and 0.3% of gastric neoplasms (47). Approximately 80% of gastric carcinoids originate in the oxyntic mucosal area of the stomach. When investigated ultrastructurally, most gastric argyrophilic carcinoids prove to be ECL tumors or have a prominent ECL cell component (29). The focus on the relationship between ECL cells and gastric argyrophilic carcinoidosis has been stimulated by the increased frequency of its detection in several pathological conditions. In particular this has been noted in patients with type A chronic atrophic gastritis (CAG) (48) and gastrinoma of the MEN I syndrome (41). In addition, the development of gastric argyrophilic carcinoids comprised of ECL cells has been noted in rodents undergoing either life-long treatment with powerful antisecretory drugs or partial fundectomy (49–54).

In recent years, the association of carcinoid tumors of the oxyntic mucosa with CAG has been noted with increased frequency. Up to 5% of patients with severe type A chronic atrophic gastritis (A-CAG) exhibit such tumors (48). Hypergastrinemia appears to be strongly correlated with the development of gastric carcinoids in the presence of A-CAG. It has been reported in patients with precursor lesions as well as in all patients in a survey of 33 published cases (48). Borch et al. (48) have noted that the levels of circulating gastrin appear to correlate positively with the degree of endocrine cell proliferation.

In Zollinger-Ellison syndrome (Z-E), a relationship between hyperplasia of argyrophilic endocrine cells and duration of the disease has been noted (3). Despite these

reports and the study of over 2000 patients with Z-E, only a few instances of gastric endocrine neoplasia have been reported (4). In contrast, in patients with Z-E as a component of multiple endocrine neoplasia syndrome type I (MEN I), there have been a number of reports of gastric endocrine neoplasia (55). This observation may be interpreted as evidence that gastric carcinoid tumors occur as a consequence of the same genetic defect as MEN I. MEN I is a rare inherited disorder in which neoplastic lesions develop either synchronously or metachronously in the endocrine pancreas, parathyroid, and pituitary. The MEN I gene has been identified on chromosome 11 by linkage studies in kindred (56–59). It is probable that clinical MEN I results from two mutational events affecting the MEN I locus and that the gene for MEN I normally functions as a growth supressor. According to the two mutation models, an inherited tumor results from the unmasking of a recessive mutation at the disease locus (60). The first mutation is carried in the germ line; while the second mutation serves to eliminate the remaining wild type allele at this locus. In MEN I patients with gastrinoma it is possible that gastrin or some other trophic factor increases the mitotic rate of the ECL cell and thereby exponentially increases the probability of occurence of the second mutational event.

Animal models of ECL cell hyperplasia

In the rat, chronic, high-dose administration of compounds that inhibit gastric acid secretion (loxtidine, tiotidine, omeprazole) are associated with the development of ECL hyperplasia and gastric argyrophil carcinoids composed of predominantly ECL cells. The development of ECL hyperplasia and neoplasia correlates with hypergastrinemia but appears independent of either the chemical structure of the compound or its cellular mechanism of action (49–51, 53, 54, 60). Larsson et al. (61) suggested that gastrin is released in the rat as a response to the increased pH caused by the inhibition of acid secretion and antrectomy has been shown to abolish the ECL cell response to acid secretion with high dose omeprazole. These results have been interpreted as evidence that a causal relationship may exist with between profound, sustained acid inhibition, hypergastrinemia, and subsequent ECL hyperplasia and neoplasia (51).

In the rat spontaneous carcinoid tumor formation is largely unknown. We have studied the mastomys, a rodent native to southern Africa, as a model for spontaneous and induced gastric carcinoid formation. In the mastomy the natural incidence of gastric carcinoid tumor formation ranges between 25 and 50% (62). The interest in the development of scientific breeding strains was initially based upon the uniform susceptibility of the mastomys to plague. Under laboratory conditions, however, it became apparent that the mastomys exhibited a high percentage of spontaneous tumors in various organs and tissues (62). Such high incidences of tumors are extremely rare in mammals other than mastomys and man. Oettle (62) first described the presence of a large number of tumors in the glandular stomach of the mastomys. Although initially identified as adenocarcinomas, subsequent work by Snell & Stewart (63) using Sevier-Munger silver impregnation identified the tumors as argyrophylic. The tumor was then reclassified as a malignant gastric carcinoid. Soga & Sato (64) in a separate study, reconfirmed this finding ultrastructurally and described specific secretory granules of an endocrine type in the cytoplasm of tumor cells.

The normal life span of a mastomys is 36–48 months (64). The tumors occur with increased frequency with age and are rarely seen in animals younger than 10 months. In one study, approximately 7% of mastomys aged 12–15 months had gastric carcinoids as compared to 52% of older animals aged 16–36 months (65).

In the rat oral administration of loxtidine, a potent H_2 blocker at a dose of 685 mg/kg/day for 116 weeks resulted in the late formation of carcinoid tumors (49). We hypothesized that inhibition of parietal cell function in the mastomys, an animal genetically predisposed to gastric endocrine hyperplasia, would promote and accelerate tumor formation. Administration of loxtidine at a dose of 1 mg/kg/day induced carcinoid tumor formation in 90% of 10-month-old mastomys (66). No tumors were found in the age-matched control animals (66).

The gastric tumors displayed typical strands or nests of tumor cells surrounded by connective tissue. Tumor cells were characterized by the presence of a variable number of neuroendocrine type cytoplasmic granules (66, 67). Immunohistochemistry demonstrated immunoreactive chromogranin and histamine in the mastomys tumor cells (65). Immunostains for gastrin, bombesin, Leu-7, neuron specific enolase, calcitonin, pancreatic polypeptide (PP) and serotonin were negative in all tumors studied with appropriate positive controls. In all gastric tumors examined approximately 30% of cells stained positively for PYY and glucagon. The labelling was confined to the cytoplasm of tumor cells and had a granular character. A slight increase in PYY and enteroglucagon-containing endocrine cells were also noted in the mucosa surrounding the tumor (67).

Gastric tumors in the loxtidine-treated animals contained no detectable gastrin (67). The tumor gastrin concentrations in the older (24 months) animals with spontaneous lesions were also below detectable limits (67). However, while plasma gastrin was elevated 10-fold in loxtidine treated animals compared with controls (66), no significant elevation in plasma gastrin was observed in tumor-bearing animals 24 months of age compared with age-matched controls without tumors (67). Nevertheless, in both loxtidine-treated animals and older animals with spontaneous tumors plasma PYY and enteroglucagon levels were elevated over 15-fold compared with controls

(66, 67). These results are consistent with the secretion of both PYY and enteroglucagon by the mastomys gastric carcinoids. Thus, in the mastomys model, since plasma gastrin is not elevated in animals with spontaneous tumors, gastrin itself cannot represent the sole common mediator of carcinoid induction.

Our results indicate that the mastomys model, due to its rapid induction rate for carcinoid tumors under the influence of H_2-receptor blockers, represents an ideal model for the study of the mechanisms involved in 'hormone-induced' carcinogenesis. Indeed, preliminary studies indicate that mastomys carcinoid tumor tissue demonstrates an increase in the expression of the c-myc and n-myc oncogenes. Thus, fundamental chromosomal and genetic alterations may be involved in the induction of gastric carcinoids in the mastomys.

Induction of ECL cell hyperplasia and subsequent gastric argyrophil carcinoidosis remains a topic of considerable concern in this age of potent gastric antisecretory therapies. Vigilant periodic reappraisal of the long-term effects of these drugs in humans is indicated to define possible increased serious pathobiological consequences. In select populations, the possibility of a neoplastic evolution of the neuroendocrine cell should be considered and monitored by regular evaluation of plasma gastrin levels and gastric biopsies. Further study of animal models such as the mastomys should allow elucidation of the cellular interactions and oncogenetic mechanisms responsible for the induction of ECL hyperplasia and eventual carcinoidosis. The perturbation of gastric secretory function by potent agents capable of altering the secretory milieu may be of considerable clinical relevance and provide a unique scientific opportunity to study certain fundamental cellular mechanisms of interaction and proliferation.

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