Differentiation of the Gastric Mucosa

II. Role of gastrin in gastric epithelial cell proliferation and maturation

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Gastrin is the principal hormonal inducer of gastric acid secretion. The cellular targets for gastrin in the stomach are the acid-secreting parietal cell and histamine-producing enterochromaffin-like (ECL) cell. Gastrin is also a growth factor, with hypergastrinemia resulting in increased proliferation of gastric progenitor cells and a thickened mucosa. This review presents insights into gastrin function revealed by genetically engineered mouse models, demonstrating a new role for gastrin in the maturation of parietal and ECL cells. Thus, gastrin regulates many aspects of gastric physiology, with tight regulation of gastrin levels required to maintain balanced growth and function of gastric epithelial cells.

IT HAS BEEN OVER 100 YEARS since John Sidney Edkin’s description of a tissue factor he termed gastrin (“gastric secretin”) that could stimulate gastric acid secretion. We now know that gastrin is the key hormonal inducer of acid secretion. Released from endocrine G cells in the antral region of the stomach in response to eating a meal, circulating gastrin stimulates acid secretion by binding to CCK-2 receptors on parietal cells and enterochromaffin-like (ECL) cells in the corpus of the stomach. Thus, gastrin stimulation of acid secretion from the parietal cell includes direct activation as well as indirect stimulation via release of the potent acid secretagogue histamine from ECL cells. In addition to being a physiological inducer of acid secretion, gastrin has been recognized for some time as a growth factor for the gastric mucosa. Hypergastrinemia stimulates mucosal proliferation leading to marked hyperplasia, including increased numbers of parietal and ECL cells. Again, it is thought that the growth factor activity promoted by gastrin likely includes both direct and indirect effects, although the mechanisms for increased proliferation of gastric progenitor cells and for changes in the census of specific cell types are not well understood. It is evident that gastrin plays a critical role in the proliferation, organization, and physiology of the gastric mucosa.

Recent studies using genetically engineered mouse models, including loss of function mutants as well as overexpression transgenics, have revealed novel functions for gastrin in the maturation or terminal differentiation of parietal and ECL cells. In this review, we focus on recent advances on the role of gastrin for gastric mucosal cell proliferation and functional maturation. We refer the reader to other recent reviews for a broader overview of gastrin function (4), regulation of gastric acid secretion (22), and involvement in gastrointestinal cancer (5).

Gastrin Stimulation of Proliferation

Increased gastric bromodeoxyuridine labeling as an indicator of proliferation has been observed in fasted rats after ingestion of a meal. This increase in proliferation is inhibited by gastrin immunoneutralization, indicating a direct role for gastrin in proliferation (20). Hypergastrinemia resulting from long-term treatment with acid inhibitors, gastrin-secreting tumors, or transgenic mice overexpressing gastrin has been shown to markedly increase gastric proliferation and mucosal thickness (5, 22). Accordingly, when gastrin levels are low or absent, such as in gastrin-deficient mice or in rats immunodepleted of gastrin, there is a modest decrease in gastric cell numbers (7, 15, 20). It has been debated, however, whether gastrin acts directly on proliferating cells or indirectly by inducing the release of local growth factors.

It is well established that both parietal and ECL cells have gastrin (CCK-2) receptors and thus could mediate the growth response induced by gastrin. In this regard, a direct mitogenic effect of gastrin has been shown in isolated rat ECL cells in culture as well as in vivo by thymidine incorporation (16, 21). Whereas there are data to indicate that gastrin directly induces the proliferation of ECL cells, no direct proliferative action on parietal cells has been shown. Rather, gastrin is thought to induce its trophic effects via the paracrine action of other growth factors such as heparin-binding EGF (1, 23, 25), amphiregulin (23, 25), and regenerating islet-derived 1 (13, 17) (Fig. 1). In addition to these established gastric growth factors, gene expression microarray approaches are starting to reveal additional gastrin-regulated growth factors produced by the stomach (10, 19). In this regard, Jain et al. (10) demonstrated that parathyroid hormone-like hormone (Pthlh) gene expression is regulated by gastrin, with a 10-fold reduction in gastrin-deficient mice. The functional significance of this recent finding remains to be tested.

Several hypochlorhydric mouse mutants have been shown to be hypergastrinemic due to a compensatory increase in gastrin to counteract the high gastric pH. Collectively, these mutant mice exhibit a thickened gastric mucosa with an increased proliferative index, as would be predicted from high concentrations of circulating gastrin (22). The importance of gastrin for remodeling the gastric mucosa has been demonstrated for two of these mutants by crossing to gastrin pathway-deficient mutants; mucosal depth was normalized in the achlorhydric H*-K*-ATPase β-subunit mutant when crossed to gastrin-deficient mice (6) and for the hypochlorhydric H2 receptor-deficient mutant when crossed to CCK-2 receptor-deficient mice (8). These studies illustrate the potent growth-promoting effect of gastrin to stimulate the gastric acid secretory system.
establishment of the major cell types, including parietal cells, deficient mice has shown that gastrin is not required for the functional maturation of these gastrin target cells. Although parietal cells in gastrin-deficient mice express the H⁺-K⁺-ATPase proton pump, a primary marker of differentiated function, the level of expression of both the α- and β-subunits is reduced in gastrin-deficient mice (10). In addition, several other parietal cell molecules thought to participate in acid secretion are reduced in expression, including the potassium channel KCNQ1 on the apical membrane and the water channel AQP4 on the basolateral membrane, suggesting that channels at both the apical and basolateral membranes of parietal cells are regulated by gastrin. Components of the parietal cell energy supply are also reduced, with decreased expression of creatine kinase B, which is proposed to supply ATP to the proton pump, as well as critical constituents of the mitochondrial electron transport chain (10). In addition to the reduced expression of several gene markers of differentiated function, the parietal cells in gastrin-deficient mice are smaller in size (9). Because parietal cells grow in size as they mature (11), a smaller size would indicate immaturity. A comprehensive microarray analysis of the transcriptome of purified parietal cells in gastrin-deficient mice showed an upregulation of a large number of Wnt and Myc target genes, suggesting a possible role of Wnt signaling in parietal cell maturation (10).

Although gastrin-induced gastric growth factors are thought to influence the cellular constitution of the gastric epithelium, they have not been demonstrated to directly stimulate the proliferation of gastric progenitor cells. In a recent study (12) using in situ RT-PCR and laser capture microscopy, gastrin receptors were identified on cells in the proliferating zone (12) suggests that gastrin may also target an as-yet-unidentified proliferative progenitor cell. Growth factors increase the proliferation of progenitor cells and can act on differentiated cells in the gastric epithelium (not shown). It is unknown whether growth factor stimulation of proliferation is direct or indirect because receptor localization in progenitor cells is not certain. Although one progenitor cell is depicted, it is possible that proliferative progenitors committed to different lineages may be differentially sensitive to stimulation by specific growth factors. Moreover, gastrin may synergize with growth factors in a progenitor cell to alter proliferation and differentiation to reshape the cellular composition of the gastric mucosa. HB-EGF, heparin-binding epidermal growth factor; Pthlh, parathyroid hormone-like hormone; Reg-1, regenerating islet-derived 1.

Immature Parietal Cells in Gastrin-Deficient Mice

Histological examination of the gastric mucosa in gastrin-deficient mice has shown that gastrin is not required for the establishment of the major cell types, including parietal cells, mucous surface and neck cells, chief cells, and endocrine (ECL) cells. However, functional analysis of parietal and ECL cells has revealed significant changes suggesting that gastrin is required for the functional maturation of these gastrin target cells (Table 1). Although parietal cells in gastrin-deficient mice express the H⁺-K⁺-ATPase proton pump, a primary marker of differentiated function, the level of expression of both the α- and β-subunits is reduced in gastrin-deficient mice (10). In addition, several other parietal cell molecules thought to participate in acid secretion are reduced in expression, including the potassium channel KCNQ1 on the apical membrane and the water channel AQP4 on the basolateral membrane, suggesting that channels at both the apical and basolateral membranes of parietal cells are regulated by gastrin. Components of the parietal cell energy supply are also reduced, with decreased expression of creatine kinase B, which is proposed to supply ATP to the proton pump, as well as critical constituents of the mitochondrial electron transport chain (10). In addition to the reduced expression of several gene markers of differentiated function, the parietal cells in gastrin-deficient mice are smaller in size (9). Because parietal cells grow in size as they mature (11), a smaller size would indicate immaturity. A comprehensive microarray analysis of the transcriptome of purified parietal cells in gastrin-deficient mice showed an upregulation of a large number of Wnt and Myc target genes, suggesting a possible role of Wnt signaling in parietal cell maturation (10).

Another indicator of loss of differentiated parietal cell function in gastrin-deficient mice is disrupted histamine signaling. The cytoskeletal adaptor protein LIM and SH3 domain protein (LASP)-1 is normally phosphorylated by PKA upon histamine stimulation. LASP-1 is markedly hypophosphorylated in gastrin-deficient mice, with reduced basal phosphorylation and loss of histamine-stimulated phosphorylation (10). The defect in histamine signaling is likely postreceptor because H₂ receptor mRNA expression is normal in gastrin-deficient mice and

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calcium signaling with histamine stimulation is seen in cultured parietal cells from gastrin-deficient mice (7, 9). The LASP-1 hypophosphorylation suggests that one or more components of cAMP signaling and/or PKA phosphorylation are abnormal in mutant parietal cells.

Although the parietal cell lifespan is unchanged in gastrin-deficient mice, migration in the gastric glands is altered, with a greater proportion of parietal cells moving toward the surface of the gastric glands in the mutant (14). Consistent with this finding is the proportional increase in parietal cells in the base of the gland in transgenic mice overexpressing gastrin (14). Thus, gastrin appears to regulate the direction of parietal cell migration along the gland axis. Because functional differences have been noted for parietal cells along the gland axis (11), the difference in migration may be relevant to cell function. Little is known about the regulation of gastric epithelial cell migration in the gastric gland. A recent cell culture study (18) has suggested that gastrin stimulates cell migration through paracrine release of EGF receptor ligands and fibroblast growth factor-1.

**Immature ECL Cells in Gastrin Mutants**

The number of ECL cells appears to be grossly normal in the mutant; however, total gastric histamine levels are reduced ~40% (7). The residual histamine content is consistent with storage in mast cells in mice (24), suggesting that ECL cell histamine is largely depleted in gastrin-deficient mice. Accompanying the reduction in histamine is a reduction in the expression of components of the histamine biosynthetic pathway. Histidine decarboxylase (HDC) gene expression and enzymatic activity are dramatically reduced in both gastrin-deficient mice and CCK-2 receptor-deficient mutants (2, 3, 7, 15). This finding is consistent with previous studies showing gastrin activation of HDC gene expression. Another aspect of the histamine pathway that is severely impaired in gastrin mutants is the extreme loss of secretory granules. Expression of the secretory granule component chromogranin A (CgA) is markedly reduced in gastrin-deficient mice (7, 15). Furthermore, analysis of ECL cell ultrastructure by electron microscopy revealed an almost complete loss of secretory vesicles in CCK-2 receptor-deficient mice (2). Moreover, the location of ECL cells in the gastric glands is altered, with ECL cells clustered nearer the base with less intermingling of ECL cells with parietal cells (7). Thus, migration of both parietal cells and ECL cells is altered in gastrin-deficient mice.

The analysis of gastrin loss of function mouse mutants shows that gastrin deficiency is associated with immaturity of the two cells primarily responsible for gastric acid secretion: parietal cells and ECL cells. Note that with the significant impairment in ECL cell histamine biosynthesis, both gastrin and histamine signaling to the parietal cell is disrupted in gastrin pathway mutants. Thus, it is not clear what aspect of the parietal cell changes are due to the loss of direct gastrin stimulation or loss of the paracrine stimulator histamine. In this regard, it is interesting that H₂ receptor-deficient mice also have smaller parietal cells and impaired acid secretion. However, the impairment in acid secretion is less severe than that described in the gastrin-deficient mice, suggesting that direct gastrin stimulation of the parietal cell is important (9).

**Improved Parietal Cell Function in Gastrin-CCK Double-Mutant Mice**

Interestingly, gastrin-CCK double-mutant mice have normal basal acid secretion despite severe functional deficits in ECL cells (3). Although parietal cells have not been critically examined in these cells, the correction in acid secretion suggests that at least some of the components for gastrin acid secretion are brought back to normal in these cells. The normalization also suggests that gastrin does not provide a unique function to the parietal cell. It is proposed that increased cholinergic vagal stimulation normalizes acid secretion in the double-mutant mice (3). Cholinergic stimulation of the parietal cell through the muscarinic 3 receptor signals predominantly through the release of intracellular calcium, similar to gastrin signaling through the CCK-2 receptor. Thus, acetylcholine stimulation may replace some aspects of gastrin function on the parietal cell. However, it is also possible that other factors, such as somatostatin, play a role in the repair seen in the double mutant, because CCK induces the acid inhibitor somatostatin, which would be lost in the double mutant (3).

**Summary and Future Directions**

The analysis of genetically engineered mouse models enables a fresh look at the well-established hormone gastrin. Analyses of gastrin-deficient and gastrin-overexpressing mouse strains have confirmed the importance of gastrin for stimulation of acid secretion, as first proposed by Dr. Edkin over 100 years ago. Moreover, this analysis demonstrates that, although gastrin is a potent growth factor for the gastric mucosa, it is not required for the establishment of the gastric glands, for differentiation of the constituent cell types, or for basal proliferation rates. However, the data show that gastrin plays a critical role for the functional maturation of the two cell types primarily responsible for acid secretion: parietal cells and ECL cells. Notably, these are the two gastric cells that contain CCK-2 receptors, suggesting that functional maturation is dependent on direct stimulation by gastrin. Parietal cells in gastrin-deficient mice are smaller and have reduced expression of various components required for acid secretion, such as the proton pump and ion channels in the apical and basal lateral membranes. Although gastrin signaling is not required for ECL cell development, it is required for ECL cells to synthesize and store histamine and for the formation of normal secretory granules. Moreover, migration of these two cells along the axis of the gland is altered. Thus, the loss of gastrin not only removes the principal hormonal inducer of acid secretion but also results in functional immaturity of the acid secretory system. This is not a permanent defect because a gastrin infusion can restore acid secretion in adult mice (7).

The mechanism for the growth factor effect of gastrin remains to be discerned. It has been known for some time that increased gastrin induces gastric mucosal proliferation, both with normal physiological responses to ingestion of a meal as well as pathophysiological responses to hypergastrinemia. Identification of gastric stem cells and progenitor cells will be critical to this understanding. In addition, genomics technology such as gene expression microarray and proteomics should help to identify new candidate growth factors and markers of the elusive progenitor cells in the stomach. The other area that demands some attention is the mechanism for cell lineage...
determination in the stomach. Significant progress is being made in this area for understanding the mechanisms driving cell fate choices in the intestine. Little is known, however, about epithelial cell development in the stomach. Understanding the fundamental mechanisms driving cell fate determination in the stomach will contribute to our understanding of mucosal cell perturbations, such as with hypergastrinemia, or during lineage changes associated with gastric cancer.

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REFERENCES

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