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REVIEW

Role of gastrin-peptides in Barrett's and colorectal carcinogenesis

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Abstract

Gastrin is the main hormone responsible for the stimulation of gastric acid secretion; in addition, gastrin and its derivatives exert proliferative and antiapoptotic effects on several cell types. Gastrin synthesis and secretion are increased in certain situations, for example, when proton pump inhibitors are used. The impact of sustained hypergastrinemia is currently being investigated. *In vitro* experiments and animal models have shown that prolonged hypergastrinemia may be related with higher cancer rates; although, this relationship is less clear in human beings. Higher gastrin levels have been shown to cause hyperplasia of several cell types; yet, the risk for developing cancer seems to be the same in normo- and hypergastrinemic patients. Some tumors also produce their own gastrin, which can act in an autocrine manner promoting tumor growth. Certain cancers are extremely dependent on gastrin to proliferate. Initial research focused only on the effects of amidated gastrins, but there has been an interest in intermediates of gastrin in the last few decades. These intermediates aren't biologically inactive; in fact, they may exert greater effects on proliferation and apoptosis than the completely processed forms. In certain gastrin overproduction states, they are the most abundant gastrin peptides secreted. The purpose of this review is to examine the gastrin biosynthesis process and to summarize the results from different studies evaluating the production, levels, and effects of the main forms of gastrin in different overexpression states and their possible relationship with Barrett's and colorectal carcinogenesis.

Key words: Gastrin; Progastrin; Glycine-extended gastrins; C-terminal flanking peptide; Hypergastrinemia; Proton pump inhibitors; Colorectal cancer; Esophageal adenocarcinoma; Barrett's esophagus

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INTRODUCTION

The polypeptide hormone gastrin was discovered in 1905 and described as a major stimulant of acid secre-

tion from the stomach antral mucosa. In the last few decades, several studies have reported on the role of gastrin in stimulating cell division and inhibiting apoptosis, suggesting that gastrin and its derivatives might promote carcinogenesis[1-5]. Gastrin and cholecystokinin (CCK) are members of a family of neuroendocrine peptides and are both physiological ligands of the CCK-B receptor (CCKBR).

Gastrin is secreted by antral G cells and interacts with the CCKBR on enterochromaffin-like (ECL) and parietal cells to induce gastric acid secretion.

Gastrin release from G cells is stimulated by the presence of food - mainly peptides[6] in the stomach, vagal release of gastrin releasing peptide, and an increase in stomach pH, as seen in achlorhydria[7]. *Helicobacter pylori* (*H. pylori*) infection is also known to cause hypergastrinemia, increasing mainly plasma levels of its amidated form gastrin-17. After eradication of the bacteria, plasma gastrin levels decrease to normal[8-10]. Gastrin release is inhibited by secretion of gastric acid, and this serves as a negative feedback control that prevents excess acid secretion. Low pH values in the stomach inhibit gastrin release by G cells, stimulating the secretion of somatostatin by antral D cells[11].

Gastrin is expressed in a variety of tissues under both normal and pathological conditions. Its main site of production are G cells from the antral mucosa, but it is also synthesized at lower levels in duodenal mucosa, fetal and neonatal pancreases, in pituitary corticotrophs, melanotrophs, and neurons, in spermatogenic cells, and in a variety of cancers.

The main products of the gastrin gene in the antrum are its amidated forms gastrin 17 and gastrin 34 (G17-NH_2 and G34-NH_2).

GASTRIN BIOSYNTHESIS

As with other peptide hormones, gastrin is synthesized initially as a large precursor molecule, which undergoes extensive post-translational modification prior to secretion. The gastrin gene spans 4.1 kb and is located on chromosome 17 (17q21). It produces a single mRNA (0.7 kb), which encodes the 101 amino acid precursor, preprogastrin[12]. Preprogastrin is translated at the endoplasmic reticulum, where the signal peptide is removed by signal peptidase, giving rise to progastrin (80 amino acids)[13]. Progastrin (PG) then progresses through the Golgi complex.

If the cell has a regulated secretory pathway, as with differentiated endocrine cells such as G-cells in the antrum, progastrin is fully processed and transported by secretory granules. It is then released by exocytosis, which is induced by secretagogues after G-cell stimulation. This is the secretory pathway of most of the amidated products, because the enzymes and conditions necessary for the processing of the immature gastrin forms are found inside secretory granules from the Golgi stack.

Progastrin is cleaved at paired amino acids by endoproteases belonging to the prohormone convertases (PC) family. PC1/3 cleavages at the dibasic sites arginine36-arginine37 and arginine73-arginine74 lead to the formation of an intermediate, which undergoes processing by carboxypeptidase E and yields glycine-extended gastrins (G-Gly) and the C-terminal flanking peptide (CTFP). The peptidylglycine α-amidating monooxygenase converts G34-gly to its amidated form and PC2 cleaves at lysine53-lysine54, producing bioactive gastrins of varying sizes (e.g., gastrin-34 and gastrin-17)[13,14] (Figure 1) .

Preprogastrin derivates can also exit the cell *via* another pathway, known as the constitutive pathway. Molecules exiting cells *via* this pathway are transported in secretory vesicles that take their contents from the Golgi apparatus and continuously fuse with the plasma membrane. Intermediate products of gastrin processing are secreted mainly by this pathway since peptides exiting this pathway do not undergo extensive posttranslational processing.

Processing and final secretion of progastrin products differ markedly depending on the expression location. In healthy adults, the main gastrin production site is antroduodenal G-cells, so the proportion of circulating gastrins depends largely on the products exiting these cells. In G-cells, the regulated secretory pathway predominates; thus, these cells mostly secrete a mixture of amidated products (95%), including G17-NH_2 (85%-90%), G34-NH_2 (5%-10%), and a mix of gastrin-14, gastrin-52, gastrin-71, and short amidated C-terminal fragments[15]. The remaining 5% of the secreted products correspond to non-amidated processing intermediates (mainly progastrin and G-Gly).

Although the majority of gastrins secreted by G-cells correspond to the amidated G17 form, peripheral blood contains almost equal amounts of G17-NH_2 and G34-NH_2 because the metabolic clearance of large gastrins is slower than for smaller forms of the peptide[16-18].

On the other hand, the proportions of the gastrin intermediates may vary in certain gastrin overexpression states, such as when proton pump inhibitors (PPIs) are used or in the presence of gastrin-producing tumors. Most of these tumors are not able to completely process gastrin, resulting in less conversion to the mature peptide[19-22].

The causes of incomplete gastrin processing during hormone overexpression are still unclear; although, it has been proposed that it might be caused by saturation of the enzymes that catalyze progastrin modifications, leading to an inability to process increasing amounts of the gene product.

Another possible reason is the lack of a well-developed regulated pathway of secretion, as in some tumor cells. In that case, progastrin exits the cell *via* the constitutive pathway directly from the Golgi terminal.

GASTRIN RECEPTORS

The actions of amidated gastrins and CCK peptides are mediated by two different receptors: CCKA and CCKB

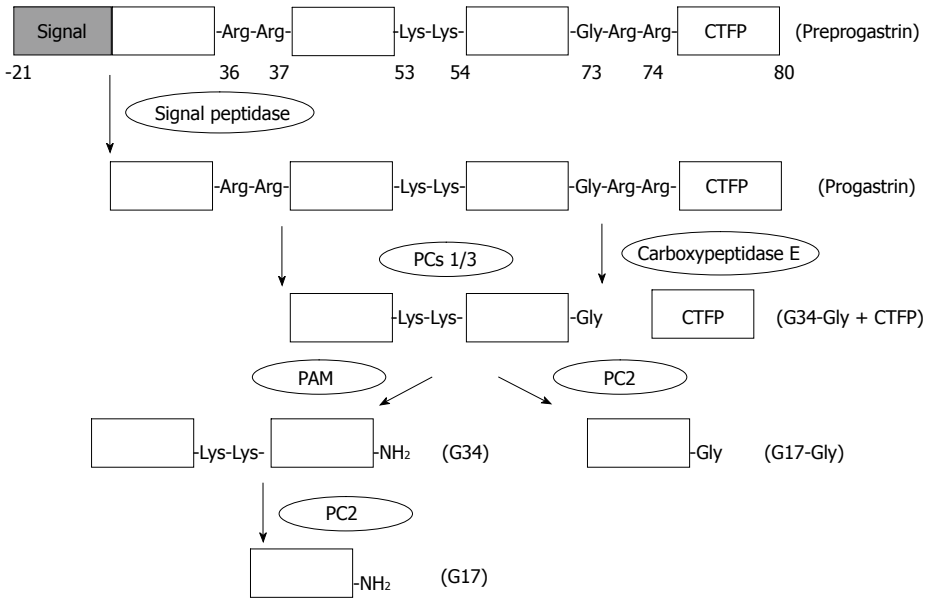

Figure 1 Main steps in preprogastrin processing in antral G cells. Arg: Arginine; Lys: Lysine; CTFP: C-terminal flanking peptide; PC: Prohormone convertases; PAM: Peptidyl-glycine α-amidating monooxygenase.

receptors, which differ pharmacologically by their affinity for gastrin (low for CCKA receptors and high for CCKB receptors)[23,24].

Gastrin and CCK peptides share a common C-terminal sequence, which has been well preserved during evolution. This conserved C-terminal active site is related to most of the known effects of these peptides, especially the tetrapeptide Trp-Met-Asp-Phe-NH_2. The specificity of the receptor binding and biological potency depends on N-terminal extensions of this common tetrapeptide.

Sulfation of the tyrosyl residue (in position six in gastrin peptides, counted from the C-terminal position, and in position seven in CCK peptides) determines the specificity for CCKA or CCKB receptors. The residue is totally sulphated in CCK peptides, so they are able to bind either CCKA or CCKB receptors with high affinity. It is partially sulphated in gastrin peptides, so they can only bind CCKB receptors.

Gastrin and CCK display similar affinities for the CCKB receptor; however, the gastrin concentration in plasma is 10- to 20-fold higher than CCK; therefore, CCKB receptors in the periphery are, in physiological terms, mainly receptors for gastrin.

The CCKB receptor has seven transmembrane domains and belongs to the superfamily of G-protein coupled receptors. CCKBR is abundantly expressed on enterochromaffin-like cells in the stomach, in the central nervous system and in some tumors, principally in the gastrointestinal tract.

Gastrin, at physiological levels, is the main mediator of meal-stimulated acid secretion. Once secreted by the antral G cells, gastrin is transported to the oxyntic mucosa of the stomach, where it interacts with the CCKBR on ECL cells, stimulating the release of histamine. Both gastrin and histamine then interact with the parietal cells, through the gastrin CCKB and histamine H_2 receptors to induce gastric acid secretion[25].

Only amidated gastrins exert their effects through CCKBR activation, while intermediate precursors such as progastrin or G-Gly interact with other receptors[3,26-28].

Most PG effects are mediated *via* the monomeric 36 kDa form of the annexin Ⅱ receptor (ANXⅡ)[29,30]. ANXⅡ is a multi-functional protein that binds acid phospholipids and actin with similar affinity. It's expressed abundantly in rejuvenating cells, but not in quiescent cells; in addition, its expression is increased in many human cancer cells, including colon and pancreatic, and it's expressed in normal intestinal epithelial cells[27,28]. ANXⅡ is absent in the brain and liver, which supports that it is only expressed in proliferating cells.

The majority of effects of G-Gly and CTFP appear to be mediated by a cellular receptor distinct from CCKBR[1,24,31-33]; yet, to date, the receptor or receptors remain unknown. Gly-G appears to be able to bind ANXⅡ, but it is still unclear whether its action is mediated *via* this interaction[1].

PPIS AND GASTRIN

PPIs are the most potent and widely used medications to reduce gastric acid secretion. These drugs are considered safe; although, some long-term side-effects have been identified, for example, all PPIs induce an increase in plasma gastrin levels. The reason for this increase remains unclear, but it may be due to the reduced activity in antral D-cells (shown by a three-fold decrease in antral somatostatin mRNA) in response to PPI-induced achlorhydria[7]. There is also an increase in plasma gastrin levels with other antacids such as H_2 receptor antagonists, but only after long-term use[34].

PPIs may induce a 2- to 4-fold increase in plasma gastrin[35,36] (mainly G17-NH_2 and G34-NH_2) with short-term treatment, whereas, in long-term therapy, some patients will develop marked hypergastrinemia (often exceeding 400 pmol/L). The antral mucosa levels of amidated gastrins and G-Gly are not affected by PPI treatment, but

Table 1 Studies assessing expression levels and/or biological effects of gastrin through its interaction with cholecystokinin-B receptor

Ref.	Specimen	CCKBR expression	G17 expression	G17 effects
Haigh *et al*[4]	Esophageal biopsies from healthy, esophagitis, BE and EAC patients; *Ex vivo* culture of BE cells; OE33(E)GR cells	CCKBR is expressed in 3/9 of healthy, 5/7 esophagitis, 10/10 BE and 7/12 EAC samples	Not assessed	G17 stimulates cell proliferation through CCKBR
Konturek *et al*[46]	Tumor and plasma samples from CRC patients; Plasma and normal colonic mucosa biopsies from healthy subjects	All the tumor samples showed CCKBR expression	CRC patients showed normal G17 plasma levels, and higher progastrin levels than healthy subjects; Celecoxib diminished plasma gastrin and progastrin levels	Not assessed
Smith *et al*[49]	Healthy colonic mucosa and colonic polyps biopsies	Normal colonic mucosa didn't show CCKBR expression; Most of the polyps analyzed showed CCKBR expression	Most of the polyps showed higher expression of the gastrin precursors than amidated forms	Not assessed
Harris *et al*[70]	Healthy esophagus and BE biopsies; OE19 and OE33 cell culture; OE21 cell culture	All three esophageal cancer cell lines express CCKBR; BE biopsies show higher CCKBR expression levels than normal esophageal biopsies	BE samples express higher gastrin levels than healthy esophageal biopsies	G17 increases activation of the antiapoptotic factor PKB/Akt, through CCKBR

BE: Barrett's esophagus; EAC: Esophageal adenocarcinoma; CCKBR: Cholecystokinin-B receptor; G17: Amidated gastrin-17; CRC: Colorectal carcinoma; OE19 and OE33 cells: Esophageal adenocarcinoma cell lines; OE21 cells: Esophageal squamous carcinoma cell line; OE33(E)GR cells: Esophageal adenocarcinoma cells permanently transfected with the human CCKB receptor.

a 6-fold increase in the antral progastrin concentration was observed after PPI therapy[37-39]. However, the gastrin levels in patients on PPIs are extremely variable, and not every subject will have a markedly increased plasma gastrin level after acid suppressive therapy.

PPIs rapidly stimulate antral gastrin secretion, but the overexpression of the gastrin gene, which is observed as increased gastrin mRNA concentrations in G-cells, is only seen after 24 h of achlorhydria[7].

To date, circulating levels of gastrin precursors have not been evaluated in response to PPI intake.

GASTRIN AND CARCINOGENESIS

As mentioned above, gastrin is a major stimulant of acid secretion in the stomach mucosa, but it also has effects in different tissues promoting cell division and inhibiting apoptosis. There is now growing evidence suggesting that elevated gastrin levels could favor the development of certain neoplasias, especially in the gastrointestinal tract[2,40-43]. To date, most of those studies have been focused on the possible relationship between elevated gastrin levels and colorectal and gastric cancers, but there is evidence that suggests a possible relationship with different tumors, even outside the gastrointestinal (GI) tract[19,20,22,44,45].

CCKBR has been observed in several tumor types, but expression of the receptor in human gastrointestinal cancers is controversial. Although some groups found CCKBR expression in many GI neoplasias[3,46], others found expression of the receptor in GI tumors only occasionally[23,29] (Table 1).

It is well established that some tumors produce their own gastrin and that gastrin can promote tumor growth in an autocrine manner[22,44,46-48], but there were conflicting findings from studies evaluating gastrin expression in tumors. This may be because initial attempts focused only on the amidated forms of gastrin. We now know that certain tumors, such as colorectal carcinoma (CRC), produce high levels of gastrin intermediates while the amidated forms are not affected[20,22]. Gastrin has been found in CRC extracts and also in adenomatous polyps[49], but not in healthy colonic mucosa. A similar pattern was found with esophageal adenocarcinoma and Barrett's esophagus (BE) (a premalignant condition that is a major risk factor for esophageal adenocarcinoma), where gastrin and its receptor were expressed at higher levels than in normal epithelium[4,50].

These observations suggest that activation of gastrin expression may be an early event in the adenoma-carcinoma or the metaplasia-carcinoma progression; thus, gastrin could favor neoplastic transformation.

Studies in animal models have demonstrated that a prolonged hypergastrinemic situation, such as in deep acid inhibition, is related to higher CRC rates and with gastric atrophy, metaplasia, gastric adenocarcinoma and carcinoid tumors[34,41,43,51] (Table 2). *In vitro* studies demonstrated that gastrin and its derivates increase the rate of cell proliferation and migration and reduce apoptosis, which are major steps in tumor development[1,3,28,44,52]. Although both *in vitro* and *in vivo* animal model studies seem to demonstrate an association between a rise in gastrin levels and a higher risk of cancer development this is still unclear in human beings. While some epidemiologic studies showed an association between, elevated gastrin levels after use of PPIs and stomach ECL and argyrophil cell hyperplasia, but couldn´t demonstrate that hypergastrinemia itself increases gastric adenocarcinoma rates[35,53-55], others found higher cancer rates (gastric and gastrointestinal overall) in hypergastrinemic patients[56,57] (Table 3).

Pernicious anemia could represent a human model

Table 2 Experimental studies in animal models exploring the impact of increased levels of gastrin peptides

Ref.	Animal model	Alteration on gastrin peptides levels	Hypergastrinemia effects
Cobb *et al*[2]	Fabp-wt mice; Fabp-mt mice	Fabp-wt mice express human PG in intestinal mucosa and Fabp-mt mice express a mutated form of human PG; Both mice show PG expression at similar levels as seen in hypergastrinemia	Mice overexpressing human PG (either the wild-type and the mutated form) are more likely to develop colonic tumors in response to AOM
Wang *et al*[5]	INS-GAS mice; hGAS mice	INS-GAS mice overexpress human amidated gastrin in the pancreatic islands; hGAS mice overexpress human PG in the liver	Both forms of gastrin showed similar proliferative effects on normal colonic mucosa
Havu *et al*[34]	Sprague-Dawley rats treated with ranitidine (2g/kg per day)	Rats showed a 3-fold increase in plasma gastrin levels	19/100 rats developed ECL carcinoids while no carcinoma was found in control animals
Watson *et al*[43]	$APC^{Min+/-}$ mice (model of multiple intestinal neoplasia) treated with omeprazole (75 mg/kg in a single oral dose)	Omeprazole increased only amidated gastrin plasma levels	PPI-induced hypergastrinemia reduced mice survival; Hypergastrinemia increased colonic adenomas proliferation; Hypergastrinemia did not increase the incidence of intestinal tumors
Ferrand *et al*[90]	MTI/G-Gly mice; hGAS mice	MTI/G-Gly mice overexpress human G-Gly throughout the gastrointestinal tract; hGAS mice overexpress human PG in the liver	Both G-Gly and PG strongly up-regulate Src, JAK2 and STAT3 activation; PG produced significantly great ERK and Akt pathways activation and TGF-α overexpression
Koh *et al*[95]	MTI/G-Gly mice	MTI/G-Gly mice overexpress human G-Gly throughout the gastrointestinal tract	Goblet cells hyperplasia and colonic hyperproliferation; Hypergastrinemia did not increase the incidence of GI tumors, but 3/10 mice developed bronchoalveolar carcinoma
Ottewell *et al*[98]	$G^{-/-}hg^{+/+}$ mice; $G^{-/-}hg^{-/-}$ mice	$G^{-/-}hg^{+/+}$ mice express human PG and no murine gastrin; $G^{-/-}hg^{-/-}$ mice do not express any forms of gastrin	PG increased colonic proliferation; PG exerts mitotic effects on colonic epithelia but does not seem to affect the small intestine epithelia

PG: Progastrin; AOM: Azoxymethane; ECL: Enterochromaffin-like cells; PPI: Proton pump inhibitors; G-Gly: Glycine-extended gastrins; JAK2: Janus-activated kinase 2; STAT3: Signal transducer and activator of transcription 3; ERK: Extracellular-signal regulated kinase; Akt: Protein kinase B; TGF-α: Transforming growth factor-alpha; GI: Gastrointestinal.

to assess effects of long-term hypergastrinemia, since it causes a long-term hypergastrinemia as a consequence of sustained achlorhydria[57]. Another human model of hypergastrinemia is Zollinger-Ellison syndrome. In this case, patients show higher rates of colonic proliferation[58], but not a higher risk for developing CRC[59].

Another study found a higher CRC incidence rate with higher serum gastrin levels[60]; while, one study found no association between PPI use and the risk of CRC[61].

It has been suggested that the discrepancy between results observed in human studies could be explained by the variability of hypergastrinemia after use of PPIs among patients[42], by differences in the duration of the follow-up period -since higher cancer rates have only been observed in long-time hypergastrinemic patients-, and by differences in the forms of gastrin being studied, given that most of the studies to date have been focused only on the amidated forms[22,62].

GASTRIN, BE AND ESOPHAGEAL ADENOCARCINOMA

Gastroesophageal reflux disease (GERD) is a chronic state in which part of the acidic stomach contents backs up into the esophagus and may cause inflammation of its epithelium. In most patients, this damaged epithelium is replaced by new squamous epithelium; however, in some subjects, this epithelium is substituted, through a metaplastic process, by an intestinal-type columnar epithelium. This condition is called BE, a premalignant state responsible for most esophageal adenocarcinoma cases (EAC). Patients with BE have a 30- to 40-fold higher risk for developing EAC than the general population[63].

In the last few decades, the incidence rates for this tumor have increased significantly[64], more than for any other type of cancer[65] in developed countries.

BE may represent a good model to study the involvement of hypergastrinemia in carcinogenesis, because frequently high levels of gastrin can be observed in BE patients. PPIs are the main pharmacological treatment for BE and the sequence of neoplastic transformation is well known.

In the pathological state caused by the damaging effects of acid contents from the stomach in the esophagus, it seems that an increase in gastric reflux pH would have a potential benefit for the patient. However, the benefits of these drugs in the management of GERD and BE are not clear. Normalization of intraesophageal pH clearly relieves gastroesophageal reflux symptoms[37], favoring differentiation and decreasing cell proliferation[66]; yet, there has been an increasing incidence of EAC in BE patients in the last few decades, despite generalized use of PPIs[67-69]. Studies addressing the potential

Table 3 Clinico-epidemiologic studies exploring the effects of proton pump inhibitors use in human beings

Ref.	Population studied	Treatment, dose and duration	Effects on gastrin levels	Physiopathological effects
Brunner *et al*[35]	143 patients with duodenal or stomach ulcer and GERD	Omeprazole 40 mg/d 1-5 yr	Plasma gastrin levels increased 4-fold after 4 mo of therapy	Hyperplasia of argyrophil cells from oxyntic mucosa; No increase in dysplasia or neoplasia rates was observed
Klinkenberg-Knol *et al*[37]	91 GERD patients	Omeprazole 20-40 mg/d 5 yr	Median serum gastrin levels increased from 60 to 162 ng/L and reached a plateau during maintenance treatment	Esophagitis symptoms ameliorated; Gastric hyperplasia rates increased from 2.5% at the beginning of the study to 20% at last biopsy
Nemeth *et al*[39]	10 patients with oesophagitis	Omeprazole 20 mg/d 6-8 wk	Plasma levels of amidated gastrins increased from 18 to 48 pmol/L; Antral levels of progastrin increased 6-fold while amidated gastrins and G-Gly remain unaltered	Not assessed
Wang *et al*[42]	82 BE patients; 13 GERD patients	All patients were on PPI therapy, once or twice daily during a median time of 74 mo	The median serum gastrin levels (40 pmol/L) was not related to the degree of dysplasia in BE	Higher serum gastrin levels were associated with high grade dysplasia and adenocarcinoma
Creutzfeldt *et al*[53]	74 patients with esophagitis or peptic ulcer	Omeprazole 40 mg/d 1-5 yr	Plasma gastrin levels increased 4-fold in 23% of patients	Patients with higher serum gastrin levels developed hyperplasia of the gastric argyrophil cells; This hyperplasia may not necessary be related to high gastrin levels
Kuipers *et al*[54]	177 GERD patients	105 patients treated with omeprazole 20-40 mg/d 5 yr; 72 patients treated with fundoplication	Not assessed	Patients treated with omeprazole and infected with H.pylori infection are at increased risk of atrophic gastritis
Lamberts *et al*[55]	74 peptic ulcer patients	Omeprazole 48 mo	Median gastrin levels moderately increased after 3 mo of therapy and reached a plateau during maintenance treatment	Significant argyrophil cell hyperplasia

GERD: Gastroesophageal reflux disease; G-Gly: Glycine-extended gastrins; BE: Barrett's esophagus; PPI: Proton pump inhibitors.

role of different molecular forms of gastrin in Barrett's carcinogenesis are discussed below.

AMIDATED GASTRINS

Amidated gastrins are, in healthy subjects, the final and most abundant product in the gastrin biosynthesis pathway. Through the interaction with their receptor, CCKBR, amidated gastrins might be involved in the neoplastic progression of BE.

Amidated gastrins and CCKB receptor expression in BE

Barrett's mucosa expresses its own gastrin. Patients with BE show higher levels of amidated gastrins than healthy subjects[44], which might be a consequence of both PPI intake and autocrine gastrin production by Barrett's mucosa[36,50]. This autocrine gastrin production diminishes with the progression to dysplasia and EAC[44], and there is not a significant difference between serum gastrin levels in GERD and BE patients[42]. The expression of its receptor increases in response to inflammation. In almost all BE biopsy samples studied, CCKBR mRNA and protein are detected; while, they are only occasionally present in healthy tissue and their presence in EAC is unclear[4,36,50,70]. In addition, expression of the receptor increases cell proliferation[26,31,44]; therefore, CCKBR may have an important role in GERD ulcer healing[36,44].

Biological effects of amidated gastrins

In vitro studies determined that amidated gastrins may promote cell proliferation and migration of BE and EAC cells, and those effects are mediated through the interaction with CCKBR[4,26,36,44].

The effects of amidated gastrins are mediated, at least partially, by the induction of cyclooxygenases (COX)-2 expression and prostaglandins production[3]. COX are membrane proteins that catalyze the limiting step in the prostaglandin synthesis pathway. Prostaglandins are molecules that may promote carcinogenesis through stimulation of cell division, induction of angiogenesis, and inhibition of apoptosis[71,72]. As a consequence of the interaction between amidated gastrins and CCKBR, COX-2 is overexpressed in Barrett's mucosa, leading to an increase in prostaglandins synthesis and cell proliferation[44,73].

COX-2 overexpression is related to the development of other GI cancers, and the use of COX-2 inhibitors, such as non-steroidal anti-inflammatory drugs is associated with a reduction in the frequency and mortality of those tumors[74-78]. *In vitro* and *in vivo* studies have shown that COX-2 inhibitors decrease cell proliferation in BE[79] and reduce the risk of developing EAC[74], suggesting that COX-2 might be a key factor in Barrett's carcinogenesis.

COX-2 overexpression seems to be an early event in the neoplastic transformation of BE. Despite the great variability observed between subjects, COX-2 levels in biopsy samples are always higher in BE mucosa than in normal esophageal epithelium[44,50,73,80].

Thus, amidated gastrins might have a role in the neoplastic progression of BE rather than in its initial development since BE cells express higher levels of gastrin, CCKB receptor and also COX-2 than EAC[44] and normal esophageal cells.

GASTRIN SYNTHESIS INTERMEDIATES: PROGASTRIN, G-GLY AND CTFP

The biological activity of gastrin synthesis intermediates was unknown until 1994[81]. Experiments carried out to determine their ability to stimulate gastric acid secretion showed negligible or less potency than fully processed amidated forms[82,83]; therefore, those investigations concluded the intermediates were inactive peptides and focused mainly on the known bioactive forms. However, in the last few decades, numerous studies have demonstrated that these molecules are far from inactive precursors. Gastrin intermediates are secreted in higher proportions than their amidated forms in certain gastrin overexpression states[39,84]; thus, knowledge of their recently known biological effects has led to several studies on these intermediates in the last few decades. To date, most of these studies have been focused on CRC; although the relative abundance of these precursors in other tissues supports that it is necessary to extend research to other organs as well.

PROGASTRIN

PG is the first gastrin synthesis intermediate after signal peptide cleavage. A study demonstrated that PG levels in antral biopsies from patients undergoing PPI treatment were up to 6-fold higher than in untreated patients[39]; although, there are currently no studies showing PG plasma levels in response to PPI administration

Progastrin expression

Studies carried out on healthy colonic and CRC tissue have shown a higher proportion of products from the early stages of gastrin synthesis (PG above all) than those from later stages (G-Gly and amidated forms) in cancer samples[20,85]. Plasma PG levels, but not amidated gastrin, are elevated in CRC patients compared with healthy subjects and those with colonic polyps, suggesting a possible tumor origin for this PG and an incomplete processing of the peptide in tumor cells[22]. Other tumors, such as pancreatic, ovarian, and lung cancer, also overexpress PG[45,47,48].

Biological effects of PG

Progastrin may exert greater proliferative effects than amidated gastrins on normal and tumor cells (CRC, pancreatic) in culture[28,86] and also has an antiapoptotic effect[87]. *In vivo* studies using mice overexpressing both G17-NH_2 and PG showed increased colonic proliferation compared to wild-type control mice. At plasma concentrations similar to those observed in certain disease states, PG can act as a co-carcinogen and significantly increases the risk for colon carcinogenesis in response to azoxymethane[2,5].

PG has negligible affinity for the receptor for amidated gastrins (CCKBR) and its effects are mediated by a different receptor: ANX Ⅱ[27-30]. This receptor is not expressed on quiescent cells and it is necessary to mediate at least 50% of exogenous PG effects on intestinal cells and more than 80% of the effects of autocrine gastrins on CRC cells[29]. ANX Ⅱ is overexpressed in human CRC and may be related to a poor prognosis[88]. It is also overexpressed in a wide variety of tumors[88,89]. The mitogenic and antiapoptotic effects of PG seem to be mediated through activation of several signaling pathways including nuclear factor-κB (NF-κB), Src, Janus-activated kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3), extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt kinases[86,90].

G-GLY

G-Gly are some of the last gastrin processing intermediates. They result from the cleavage of the C-terminal arginyl residues of progastrin by carboxypeptidase E, before the amidation step.

G-Gly expression

As with other gastrin intermediates, there are few studies focused on assessing possible changes in plasma or tissue levels of G-Gly in different situations. It seems that PPIs don't significantly affect its levels because antral mucosa levels of G-Gly remain unaltered after treatment with PPIs[39]. In addition, CRC doesn't alter plasma G-Gly concentrations[22]; while, tumor biopsies and cell lines derived from CRC show higher levels than healthy tissue[20,22]. G-Gly levels are also increased in the gastric mucosa from patients with gastrinoma[91]. Outside the GI tract, only a small proportion of lung cancer cases analyzed showed G-Gly overexpression, which was inversely related to survival rates[45].

Biological effects of G-Gly

Even small variations in the levels of G-Gly may affect proliferation and apoptosis. G-Gly effects can be observed at concentrations at least one order of magnitude less than for amidated forms[33,92]. G-Gly can act as a growth factor for many cultured cells, including gastric, pancreatic, colonic cancer cells, and non-transformed cells[33,52,81,93,94]. It also decreases apoptosis in CRC and EAC[1,92] cells and increases migration in CRC cells[40]. In vivo experiments using transgenic mice overexpressing this intermediate demonstrated that higher G-Gly levels are related to colonic hyperproliferation, but were not

able to cause tumors alone[90]. Surprisingly these experiments showed higher bronchoalveolar cancer rates in the animals overexpressing the molecule[45,95].

The proliferative effects of G-Gly are dependant, at least partially, on COX-2 expression in EAC cells because the use of COX-2 inhibitors abolishes the proliferative effects of the molecule[52].

To date, the G-Gly receptor remains unknown but the majority of its effects seem to be mediated *via* a different receptor than CCKBR[1,33,96]. Although G-Gly has been shown to bind to ANX Ⅱ, no effects were observed[29]. The G-Gly interaction with a receptor distinct from CCKBR leads to JAK2/STAT3, Akt, NF-κB, PI3k, and ERK activation[52], increasing COX-2 expression. In contrast, the antiapoptotic effects of G-Gly occur independently from COX-2 expression[1].

CTFP

CTFP is the gastrin synthesis intermediate generated after cleavage of progastrin into its dibasic residues, generating G-Gly and the 6-amino acid CTFP. After the discoveries that the gastrin intermediates PG and G-Gly are present in normal antrum and certain tumors[20-22,48] and have effects enhancing cell proliferation and inhibiting apoptosis[5,28,29,33,52], several studies focused on those molecules. However, little attention has been paid to CTFP.

CTFP expression

In plasma and human antral extracts from healthy subjects, CFTP is the most abundant peptide (four-fold higher than the next most abundant peptide in antral samples and 30-fold higher in plasma). In CRC patients, CTFP levels are elevated in tumor mucosa and remain unaltered in plasma[32].

CTFP biological effects

CTFP effects have been tested on colonic and gastric cancer cell lines *in vitro*. CTFP showed higher potency in stimulating cell growth than G-Gly in colon tumor cells and a similar potency in gastric cancer cells. CTFP also stimulated cell migration in a non-transformed mouse gastric cell line and activated MAPK phosphorylation in colon cancer cells *via* a different receptor than CCKBR[32]. CTFP also exerts anti-apoptotic effects[97]. To date, only a few studies have focused on CTFP, but it has been determined that it is a biologically active molecule that is secreted in higher amounts than any other product of the gastrin gene. Thus, further experiments with this peptide should be carried out.

CONCLUSION

In summary, data derived from *in vitro* studies and animal models strongly suggest that high levels of gastrin may exert carcinogenic effects, on BE and colorectal epithelia, but also elsewhere. In addition, certain tumors produce their own gastrin, which might contribute to support tumor growth. However, it is currently not clear if high gastrin levels have the same effects in human beings. Most studies have been focused on amidated gastrins. Although, intermediates of gastrin synthesis can exert even greater carcinogenic effects than the amidated forms and in certain situations they become the most abundant forms of gastrin. Therefore, more studies evaluating these molecules are needed to elucidate the potential role of gastrins in human carcinogenesis.

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