Gastrin is the main hormone stimulating gastric acid secretion, but it exerts proliferative and anti-apoptotic actions on various cancer cell types, in addition to its well-known trophic effect on enterochromaffin-like cells. As treatment with proton pump inhibitors (PPIs) increases the biosynthesis and secretion of gastrin, it has been postulated that treatment with PPIs could increase the risk of cancer, especially in Barrett’s esophagus, gastric carcinoids, and colorectal cancer (CRC). Some tumors produce gastrin of their own, which can act in an autocrine manner to promote tumor growth. In addition, gastrin is known to foster the tumor microenvironment. However, in spite of these potentially increased cancer risks due to PPI-induced hypergastrinemia, prospective, large-scale cohort studies did not show an increase in CRC prevalence. The question as to why the long-term use of PPIs was not associated with an increased cancer risk of CRC might be answered by the fact that the PPIs antagonized the trophic effects of hypergastrinemia. Furthermore, the blockade of proton pumps or potassium channels in cancer cells could limit the abnormal glycolytic energy metabolism of cancer cells. Apart from their suppressive effect on gastric acids, PPIs exert an anti-tumor effect through the selective induction of apoptosis as well as an anti-inflammatory effect, and they protect cells from developing chemo- or radio-therapeutic resistance. Moreover, the anti-carcinogenic actions of PPIs were augmented with PPI-induced hypergastrinemia. Together with their potential targeted killing of cancer stem cells, these effects demonstrate their potential anti-cancer actions.

**Key words:** gastrin, proton pump inhibitors, colorectal cancer, trophic action, anti-carcinogenesis, cancer prevention, Warburg effect
Fig. 1. Gastrin promotes inflammation or fosters the tumor microenvironment. (A) and (B) RT-PCR showed an increased expression of MMP3, MMP9, COX-2, VEGF, and IL-8 in macrophage cells at 24 h after administration of gastrin. Stromal cell-derived factor-1alpha (SDF-1α, now known as C-X-C motif chemokine 12 (CXCL12)) a chemokine that is encoded by the CLCL12 gene in humans and is implicated in the chemotaxis of lymphocytes, showed an increased expression after administration of gastrin. However, co-treatment of gastrin and PPI did not increase SDF-1α expression. SDF-1α is a small cytokine belonging to the intercine/chemokine family, members of which activate leukocytes and are often induced by pro-inflammatory stimuli such as lipopolysaccharide, TNF-α, or IL-1β, indicating that gastrin contributes to fostering the tumor microenvironment, in addition to its authentic proliferative effect. (C) and (D) Gastrin significantly increased APC mutation-induced intestinal polyposis in APC<sup>Min/+</sup> mice, whereas a significant decrease in intestinal polyposis was seen in APC<sup>Min/+</sup> mice treated with PPI, in spite of hypergastrinemia.
Cancer, mediated via CCK-2R (11). Some tumors also produce gastrin that can promote tumor growth in an autocrine manner, and certain cancers are extremely dependent on gastrin to proliferate (12). In a study on CRC, gastrin significantly promoted cell growth by inducing the expression of cyclooxygenase-2 (COX-2) and the release of prostaglandin E2 (PGE_2) in a rat intestinal epithelial cell line, via the epidermal growth factor receptor pathway (13). CRC cells aberrantly produce gastrin and exhibit increased expression of its receptors. Furthermore, gastrin and its receptors modulate the function of immune cells and fibroblasts in cellular stress, injury, and repair, as well as during cancer progression (14). According to a previous report (13), gastrin promoted intestinal polyposis through either direct gastrin receptor-mediated proliferative signaling or by stimulating the tumor microenvironment through macrophage activation and subsequent chemotaxis (Fig. 1A). By using a murine cytokine antibody array designed for simultaneous detection of 62 cytokines, IL-1β, IL-3Rb, stromal cell-derived factor-1α (SDF-1α also known as CXCL12), thymus and activation-regulated chemokine (TARC)/chemokine (C-C motif) ligand 17 (CCL17), and T-cell activation-3 (TCA-3) were identified to be significantly increased in macrophage cells treated with gastrin (Fig. 1B). All of these proteins have been reported to be implicated in tumor angiogenesis as well as in platelet chemotaxis, allergic reaction, and macrophage-derived sepsis (15-17). In another report on the association of hypergastrinemia with pancreatic cancer, CCK and/or gastrin were found to be produced endogenously in established cancer and to stimulate the synthesis of more receptors and tumor growth by an autocrine mechanism, and CCK was hypothesized to potentiate carcinogenesis by interplay with inflammation in the pancreatic microenvironment (18). However, as CCK actions might not be the result but an early promoter of cancer, the authors considered the causality dilemma between the levels of gastrin and biomarkers of inflammation (19). The authors also investigated the role of gastrin in mucosal inflammation in Helicobacter pylori-infected Mongolian gerbils using a gastrin receptor antagonist, and found that the antagonist prevented Helicobacter pylori-induced gastritis (24). In Helicobacter pylori-infected gerbils, a high salt diet was shown to significantly elevate the serum gastrin level and inflammatory cell infiltration in a dose-dependent manner (25). Gastrin induced COX-2 expression in gastric epithelial cells (26) and in gastric cancers in Helicobacter pylori-infected humans (27), and a close relationship was found between the levels of gastrin and biomarkers of inflammation including interleukin-8 (IL-8), human leukocyte antigen (HLA) class II molecules, and reactive oxygen species (28). Taken together, these findings indicate that gastrin is strongly associated with the inflammatory reaction of the gastric mucosa in response to Helicobacter pylori infection. In reverse, various cytokines including IL-1, IL-2, IL-6, IL-8, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) play an important role in gastrin production and secretion.

Gastrin promotes the interaction between human leukocytes and endothelial cells through the endothelial expression of P-selectin and vascular cell adhesion protein 1 (VCAM-1) (29). Singh et al. (30) recently phrased some paradigm shifting concepts in the understanding of the link between gastrin and CRC as follows: 1) gastrin exerts anti-apoptotic and hyperproliferative effects on colon epithelial cells, 2) colon cancers increasingly express the gastrin gene as the disease progresses through the hyperplasia-adenoma-carcinoma sequence, 3) constitutive over-expression of gastrin may transform stem cells, and 4) gastrin augments the risk of CRC in patients who are positive for other risk factors such as obesity and inflammatory diseases, which was the most persuasive concept. Gastrin exerts inflammatory effects that induce pro-inflammatory cytokines, chemokines, and adhesion molecules in diverse ways. Particularly the cells and mediators of inflammation form a major part of the epithelial tumor microenvironment (31). Inflammatory mediators such as COX-2, inducible nitric oxide synthase (iNOS), IL-1β, and IL-6 were all increased in inflammatory bowel disease and CRC after gastrin stimulation. Although several studies have indicated that gastrin potentiates carcinogenesis through fostering the interplay between inflammation and the gastrointestinal tumor microenvironment and contributes to hormone-dependent gastrointestinal carcinogenesis, further investigations are required to elucidate the exact mechanism by which gastrin exerts these effects (Fig. 1C). Apart from the above-mentioned mediators, gastrin-releasing peptide receptor (GRPR) was...
reported to be implicated in the inflammatory response in the gastrointestinal tract, indicating that GRPR is a potential therapeutic target for inflammatory diseases (32).

PROTON PUMP INHIBITORS INDUCE HYPERGASTRINEMIA, BUT EXERT ANTI-TUMOR EFFECTS BEYOND AUTHENTIC ACID SUPPRESSION

PPIs are currently among the most widely used drugs to control gastric acid-related diseases, and are frequently used for long-term treatment as the benefits outweigh the side effects in most patients (33). In response to the acid suppression by PPIs, G-cells increase their gastrin production in an attempt to promote acid secretion. Gastrin stimulates acid secretion by two mechanisms: by direct action on the CCK-B receptors in the basolateral membrane of parietal cells, and indirectly by affecting the CCK-B receptors of ECL cells that respond by releasing histamine, which stimulates parietal cell acid secretion by binding to the histamine type 2 receptor (34-36). Because long-term use of PPIs is becoming increasingly common, the (side) effects of chronic acid suppression on gastrin production, and the potentially associated risks of developing hypergastrinemia and/or certain cancers, have been studied extensively. Despite the hypergastrinemia-inducing effects of PPIs or histamine type 2 receptor antagonists, a recent cohort study showed that long-term therapy with regular doses of PPIs did not increase the risk of gastrointestinal cancer (37). Although animal studies clearly showed that hypergastrinemia increased the incidence and proliferation rate of epithelial neoplasms in colon cancer (4, 13), the prevailing hypothesis has been that in humans, the inhibition of gastric acid secretion is not sufficient to induce tumors (38). Two possible explanations for this unexpectedly low incidence of gastrointestinal cancer in spite of hypergastrinemia in patients have been proposed. The first was with regard to the double-edged sword-like role of gastrin in carcinogenesis; while gastrin suppresses the growth of CCK-2R-expressing cancer cells by inducing apoptosis in vitro (39), it also has trophic effects on gastrointestinal carcinogenesis (13). The second was that PPIs might directly inhibit hypergastrinemia-associated carcinogenesis. We previously demonstrated that long-term administration of PPI prevented colitis-associated carcinogenesis by exerting anti-inflammation, anti-oxidation, and anti-mutagenesis effects (40).

PPIs have anti-oxidant properties and direct effects on neutrophils, monocytes, endothelial cells, and epithelial cells that might prevent inflammation beyond the authentic gastric acid-suppressive action, through blocking the efflux of hydronium in parietal cells. PPIs may mediate anti-inflammatory effects through the inhibition of angiogenic growth factors (41) and of the expression of intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 as well as of endothelial-dependent neutrophil adhesion (42), and through the selective induction of apoptosis in cancer cells (43) (Fig. 1D). Apart from the P-type ATPase proton pumps existing in parietal cells, cancer cells show a metabolic shift that makes them overproduce protons, which potentially disturbs the cellular acid-base homeostasis (41). Various ATPases including vacuolar-type (V-type) and mitochondrial-type (M-type) ATPases are involved in the control of the cytosolic pH and in the generation of proton gradients (positive inside) across cellular membrane systems such as the Golgi complex, endosomes, and lysosomes. Similar to P type-ATPase PPIs, v
type-ATPase inhibitors may induce tumor cell apoptosis (44). Furthermore, PPI may reduce tumor resistance to chemotherapeutics (45). Pretreatment with PPIs sensitized tumor cell lines to cisplatin, 5-fluoro-uracil, and vinblastine, and increased the extracellular pH, as well as the pH of lysosomal organelles, which was consistent with the cytoplasmic retention of the cytotoxic drugs or, in the case of doxorubicin, with targeting to the nucleus. The manipulation of the extracellular and/or intracellular pH in tumors by treatment with PPIs seems to have a considerable therapeutic potential for cancer therapy. Clinical data will provide the proof-of-concept on the use of PPIs as a new class of anti-tumor agents with a very low level of systemic toxicity in comparison with standard chemotherapeutic agents (46). All of these new findings with regard to PPIs as blocking agents of the Warburg effect were summarized in Fig. 2.

A recent study reported that treatment with PPIs aggravated the intestinal damage induced by non-steroidal anti-inflammatory drug (NSAID) (47). The results indicated that suppression of gastric acid by PPIs reduced the number of intestinal Actinobacteria (Bifidobacteria spp.), and subsequently induced dysbiosis in rats. Simultaneous administration of commensal bacteria during PPI treatment alleviated the NSAID-induced intestinal injury (47). Colonization of germ-free mice with jejunal bacteria from PPI-treated rats increased the NSAID-induced intestinal damage in comparison with mice colonized with bacteria from vehicle-treated rats (47), suggesting that the prevention of dysbiosis can reduce the severity of NSAID-induced enteropathy (47, 48).

PARADOXICAL AUGMENTED ANTI-CARCINOGENIC ACTION BY THE COMBINATION OF PROTON PUMP INHIBITORS AND HYPERGASTRINEMIA

Our current investigations suggest a paradoxical anti-carcinogenic action of PPI and hypergastrinemia in spite of the trophic effect of hypergastrinemia on various GI cancers. Although the serum gastrin levels in mice increased five-fold upon administration of PPIs, long-term omeprazole treatment prevented colitis-associated carcinogenesis (40). In addition, clinical follow-up studies revealed that their long-term use of PPIs did not significantly increase the prevalence of CRC (37, 49), supporting our finding that PPIs could abolish or cooperate with hypergastrinemia to inhibit the otherwise proliferative action of increased levels of gastrin. Therefore, we hypothesized that PPIs might antagonize the trophic action of gastrin or synergize with gastrin to exert augmented anti-carcinogenic actions. Our in vitro and in vivo studies using an APC/Min+ mouse model showed that co-administration of PPIs and gastrin significantly inhibited intestinal

![Fig. 3. Summary of the anti-carcinogenic actions by PPI. As seen in Fig. 1C and 1D, paradoxically augmented antitumor effects by the combination treatment with PPI and gastrin led to attenuated intestinal polyposis in APCMin/+ mice. Gastrin administration significantly accentuated intestinal polyposis in APCMin/+ mice, whereas intestinal polyposis was significantly reduced in APCMin/+ mice with the combination of PPI and gastrin. The mechanism by which PPI could antagonize the intestinal polyposis-promoting action of gastrin in APCMin/+ mice is described.](image-url)
polymer either via the suppression of β-catenin-associated proliferative signaling, or via the attenuation of gastrin-promoted inflammation (Fig. 3), suggesting that PPI and gastrin might have a synergistic effect in blocking intestinal polyposis (50). This raised the question as to how PPIs blocked the gastrin-promoted proliferative effects in intestinal polyposis. Co-treatment with PPIs and gastrin significantly decreased nuclear translation and activation of β-catenin and increased apoptosis, compared to treatment with PPIs alone (51). In addition, the expression of cell cycle-associated factors, such as cyclin D1, cyclin E, CDK2, and CDK4 was significantly decreased in vivo upon treatment with PPIs and gastrin in the APCmin mouse model (51).

In addition, the combined treatment with PPIs and gastrin showed an anti-carcinogenic action through the inhibition of angiogenic processes including the activation of CD31 and mitogen-activated protein kinase (MAPK) and inflammatory signaling (51). Treatment with PPIs and gastrin decreased the phosphorylation of p38, extracellular signal-regulated kinases (ERK), and c-Jun N-terminal kinase (JNK) compared with treatment with PPIs alone. TNF-α and COX-2 are known to be involved in intestinal polyposis as well as colon cancer (51, 52), and their expression significantly decreased upon the combined treatment. The level of PGE2, a product of COX-2, decreased upon the combined treatment compared with treatment with PPIs alone. We tested the hypothesis that the anti-proliferative effect of PPIs was through antagonism of the trophic effect of gastrin, and showed that the binding of gastrin to the CCK-B receptor indeed inhibited the promoter activity of the Tcf/Lef transcription factors downstream of β-catenin. For the first time, the inhibitory action of PPIs on the binding of gastrin to the CCK-B receptor was demonstrated, which was in accordance with a previous report on the fact that PPIs can modulate action the Warburg effect (53). A recent study on the synergistic effect of PPIs and doxorubicin on solid tumors showed that an increased endosomal pH caused by PPI treatment resulted in an increased uptake of doxorubicin by the tumor cells, demonstrating the clinical potential of doxorubicin in the treatment of solid tumors (54).

GASTRIN, PROTON PUMP INHIBITORS, AND CANCER STEM CELLS: DO THEY CONTRIBUTE TO CANCER THERAPY OR TO CHEMOQUIESCENCE?

Cancer stem cells (CSCs) have been identified as rare cell populations in many cancers and are capable of self-renewal and differentiation into various types of cancer cells (55). They are responsible for cancer initiation, progression, metastasis, recurrence, and drug resistance. Therefore, research efforts have been focused on the development of CSC-related therapies and the identification of the key molecules controlling these CSC populations to achieve a higher cancer treatment success-rate (56). The clinical relevance of CSCs has been strengthened by the fact that CSCs are resistant to conventional chemotherapy and radiation treatment and are very likely to be the origin of cancer metastasis. A number of cell surface markers such as CD44, CD24, and CD133 are often used to identify and enrich CSCs (57). Therefore, targeting cells harboring these biomarkers could improve cancer treatment outcome and patient survival (58). In addition, targeting mitochondrial bioenergetics, which was shown to induce caspase-independent cell death in a panel of CSCs, could overcome some limitations of current cancer therapies (59).

In a recent publication by Patel et al. (54), the PPI pantoprazole was used to modify the distribution and activity of doxorubicin as a potential strategy to improve the therapy of solid tumors, because the limited distribution of drugs within solid tumors is an important cause of drug resistance. Doxorubicin may be sequestered in acidic organelles, thereby limiting drug distribution to distal cells and diverting drugs from their target DNA. Treatment with pantoprazole affected doxorubicin uptake, as well as its distribution and activity (55). In addition, PPIs can modulate the Warburg effect in tumor cells (60), as they modify anaerobic glycolysis and ATPase binding cassette (ABC) transporters, rendering tumor cells more sensitive to chemo- or radio-therapy, and they induce apoptosis. Huang et al. (61) recently reported that both ATP-binding cassette sub-family G member 2 (ABCG2) and v-type-ATPase were over-expressed in esophageal squamous cancer cells during different stages of the cancer, suggesting that they have an interactive relationship. In addition, ABCG2 and v-type-ATPase expression may be strongly associated with drug resistance and tumor metastasis. Whittaker-Menezes et al. (62) have recently proposed a new model to explain energy transfer in cancer metabolism, in which cancer cells behave as metabolic parasites, extracting nutrients from normal host cells such as fibroblasts, triggered by the secretion of hydrogen peroxide. Therefore, we hypothesize that PPIs might stimulate parasite-like behavior in cancer cells through the regulation of ABC transporters leading to apoptosis, mitophagy, and selective CSC surveillance through oxidative phosphorylation (63) (Fig. 2).

Apart from their effects on CSCs, PPIs can affect the metabolism of bone cells, suggesting an association between PPIs and osteoporosis. Costa-Rodrigues et al. (64) investigated the concentration- and time-dependent effects of the PPIs omeprazole, esomeprazole, and lansoprazole on various human osteoclast precursor cells and concluded that PPIs might have a direct deleterious effect on these cells, resulting in decreased bone turnover. Interestingly, induced pluripotent stem cells share some basic properties with cancer cells such as self-renewal and pluripotency, as well as several metabolic alterations such as the glycolytic or Warburg phenotype (65). Therefore, PPIs might selectively induce cancer cell death and cause augmented apoptosis in PPI-induced hypergastrinemia (40, 66). In addition, PPIs might be used for targeting CSCs and the re-routing of somatic cells to pluripotency. Shin et al. investigated the mechanism of resistance to 5-fluoro-uracil in colorectal cancer. They found that the ATP synthase inhibitor oligomycin A strongly antagonized the 5-fluoro-uracil-induced suppression of cell proliferation, and that ATP synthase down-regulation is a bioenergetic signature of colorectal carcinomas (67). Recently, Jinesh et al. reported a link between CSCs apoptosis and proton pumps in cancer cells (68). They demonstrated that apoptotic blebs of cancer cells fused together to form “blebbishields,” which fuse further to form spheres. The phenomenon was shown to involve active caspases and N-linked glycosylation. Blebbishields displayed transiently down-regulated expression of stem cell markers and the sphere-forming blebbishield-derived cells were carcinogenic, demonstrating that CSCs survived apoptosis by blebbshield and sphere formation. Sphere formation was enhanced by acidic pH and was counteracted by PPIs, resulting in selective cell death of CSCs with PPI.

We are currently developing nanoparticle-PPI complexes for the more efficient delivery of the PPIs to tumor cells, preferably the CSCs. Targeting of the CSCs is essential for developing novel therapies to prevent cancer relapse and the emergence of drug resistance, and nanocarrier-based therapeutic agents, nanomedicines, and nanoparticles have been used to achieve longer circulation times, and better stability and bioavailability than current therapeutics. Zhao et al. (69) have reviewed successful applications of nanomedicines to target CSCs for eliminating tumors and preventing their recurrence. Recently, Pardo and Stuhmer (70) reported that potassium channels can serve as therapeutic targets in cancer patients, which suggests that the regulation of either proton pumps or potassium channels, which are both implicated in acid suppression by hypergastrinemia, can be future targets for cancer control.
In this review article, we described the putative antagonizing effect of PPIs against the trophic effect of gastrin in hypergastrinemia, resulting in a seemingly paradoxically anti-carcinogenic effect. Furthermore, blocking of the ABC or potassium channels in cancer cells could lead to chemokinesecence or cancer prevention. In future, the new insights on the joint anti-carcinogenesis effect of PPI and gastrin, together with the further elucidation of the mechanism by which PPIs can specifically target CSCs and the development of the nanoparticle-PPI complexes, will allow specific targeting of CSCs and open up new approaches to cancer treatment.

 Abbreviations: BE, Barrett’s esophagus; CCK-2R, cholecystokinin-2 receptor; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase-2; CRC, colorectal cancer; CSCs, cancer stem cells ECL cells, enterochromaffin-like cells; ERK, extracellular signal-regulated kinases; GRPR, gastrin-releasing peptide receptor; HLA, human leukocyte antigen; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NSAIAD, non-steroidal anti-inflammatory drug; PGE2, prostaglandin E2; PKB, protein kinase B; PPIs, proton pump inhibitors; SDF-1α, stromal cell-derived factor-1α; TARC, thymus and activation-regulated chemokine; TCA-3, T-cell activation-3; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion protein 1.

Y.-M. Han, J.-M. Park equally contributed to the study.

Acknowledgements: This work was supported by a grant from the Ministry of Education and Science Technology (2010-0002052), Korea, and by the JSPS Asian CORE Program, Japan. This article is based on our presentations at the 1st Global Gastrointestinal Club of Experimental Biology/IUPHAR GI Section of ASPET "The best of my research: The last 10 years” meeting organized in Boston 2013 by Prof. Sandor Szabo, University of California, Irvine and GI Section VA Medical Center, Long Beach, California, USA.

Conflict of interests: None declared.

REFERENCES


29: 6581-6590.


66. Han YM, Park JM, Park SH, Hahn KB, Hong SP, Kim EH. Gastrin promotes intestinal polyposis through cholecystokinin-B receptor-mediated proliferative signaling


Received: December 26, 2013
Accepted: January 9, 2015

Author’s address: Prof. Ki Baik Hahm, M.D., Ph.D., CHA Cancer Prevention Research Center, CHA Bio Complex, Sampyo-dong, Pangyo, and CHA University Bundang Medical Center, Seongnam, Korea 135-081.

E-mail: hahmbk@cha.ac.kr