Gastrointestinal Hormones and Cell Proliferation

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Abstract: There is no question that gut peptides are trophic for normal gut mucosa. Gut peptides can function in an endocrine, paracrine or autocrine fashion. We examined the effects of gut peptides on the growth of animal and human cancers of the gastrointestinal (GI) tract and pancreas in vivo and in vitro. We also examined the role of growth factors and bioamines in the regulation of growth of human endocrine tumors. Our studies have shown that gut peptides (gastrin, VIP, neurotensin, and bombesin) regulate growth of some cancers of the GI tract and pancreas. We have found that peptide action is mediated through specific receptors and that cell-specific differences in receptor expression occur. We have also begun to examine the intracellular signal-transduction pathways involved in endocrine and autocrine actions of these peptides on growth of GI cancers. We have found that cell-type-specific differences exist among the various signal-transduction pathways (cyclic AMP, phosphatidylinositol hydrolysis (PI), intracellular calcium ([Ca^{2+}]i) mobilization, and tyrosine phosphorylation) and that different receptors for the same hormone may be linked to different signal-transduction pathways depending upon cell type. We have also found that autocrine growth regulation of human pancreatic carcinoid occurs through specific receptor-mediated signal-transduction pathways. We will discuss the mechanisms of action and potential therapeutic uses of manipulation of gut hormone levels or hormone antagonists to inhibit the growth of GI tract cancers.

Effect of Gastrin on Growth of Cancer Cells In Vivo

The trophic effect of gastrin on transplantable rodent and human colon cancer in vivo has been well documented.1-4 We have reported that pentagastrin stimulated cancer growth and decreased survival of mice inoculated with MC-26 mouse colon cancer cells.1 The effect of gastrin on growth of colon cancer is most likely mediated by the interaction of gastrin with its cellular membrane receptor. The trophic effect of gastrin on MC-26 cells is mediated through the interaction of gastrin with specific, high-affinity gastrin receptors on MC-26 tumor cells.5 In non-pentagastrin-treated mice, the binding affinity of the gastrin receptor on tumor membranes significantly decreased as tumor growth progressed. On the other hand, both the binding affinity and gastrin receptor levels of tumor membranes were maintained by pentagastrin treatment. These studies demonstrated that the trophic effects of gastrin on MC-26 cells are probably mediated by its regulation and maintenance of the gastrin receptor on the cancer cells.6

Based on these studies, we evaluated the effects of lowered levels of endogenous gastrin on growth of colon cancers.7 Enprostil is a synthetic prostaglandin E2 analogue that suppresses the postprandial release of gastrin.8 We found that enprostil treatment of mice inoculated with MC-26 colon cancer cells significantly reduced both fasted and food-stimulated gastrin levels and inhibited growth (size and rate) of MC-26 colon cancers. We have, furthermore, examined the effect of a gastrin receptor antagonist, proglumide, on the growth of MC-26 colon cancer.9 Tumor size was significantly reduced and survival was significantly prolonged in proglumide-treated mice compared with controls.10 These results established that endogenous gastrin is important for the growth of MC-26 colon cancer. These and other studies show that endogenous gastrin is an important regulator of the growth of colon cancers which possess gastrin receptors.

The response of GI tumor growth to a specific peptide hormone cannot be predicted based on the presence of a receptor for that specific hormone. Shown in Fig. 1 is the response of three human tumors to caerulein, a cholecystokinin analog. Caerulein was
trophic for the human pancreatic adenocarcinoma (SKI) which possesses cholecystokinin (CCK) receptors, and it had no effect on a pancreatic carcinoma (CAV) which lacks CCK receptors. However, the growth of a human cholangiocarcinoma (SLU-132), which possesses a high-affinity CCK receptor, was inhibited by caerulein. Therefore, GI hormones (GIH) may either stimulate or inhibit the growth of GI and pancreatic cancer through interaction with a specific cell-surface receptor.

Effect of Gastrin on Growth of Colon Cancer Cells In Vitro

The direct effects of gastrin on established human colon cancer cell lines have been extensively studied in vitro, and the majority exhibited increased growth in response to gastrin. Some cell lines responded to gastrin only under specific conditions, for example, synchronization of cells, low serum conditions, or early culture of cells. The growth of three human colon cancer cell lines, LoVo, COLO 320, and HT-29, was stimulated by gastrin in a dose-dependent, nonlinear fashion. The effective dose of gastrin for stimulation of growth was dependent on cell line (LoVo: $10^{-12}$ and $10^{-8}$ M with a nadir at $10^{-10}$ M; COLO 320: $10^{-10}$ and $10^{-6}$ M with a nadir at $10^{-9}$ M; HT-29: $10^{-7}$ M of gastrin). However, the growth of another human colon cancer cell line, HCT116, was inhibited by gastrin in a similar fashion. Finally, the growth of other human colon cancer cells, WiDr, Caco-2, and LS180, was not affected by gastrin. The reason for the nonlinear response to gastrin is still unclear. It may be that the cell lines are heterogenous populations, some of which may have no gastrin receptors or have gastrin receptors with different sensitivities to gastrin. The most important finding of these studies is that a low serum concentration and a broad dose-range of gastrin must be employed to properly assess the effect of gastrin on growth of colon cancer cells.

To express their growth-regulatory effect, peptide growth factors must bind to their specific receptors on cell membranes. GIH receptors are membrane-bound glycoproteins which fulfill three primary purposes: (1) they recognize the ligand at extremely low concentrations of the peptide (affinity), (2) they are able to recognize and bind only its ligand among a vast number of other extracellular molecules (specificity), and (3) they translate the peptide-receptor interaction into a series of intracellular events eventually leading to a biological response (signal transduction). The amino-acid structure of all GIH receptors, including the gastrin receptor (GR), indicates that they are coupled to the second messenger effector systems by glycoproteins and thus may utilize the signal-transduction pathways of cyclic AMP, PI hydrolysis and Ca²⁺-calmodulin.

The affinity, size and tissue distribution of the GR was evaluated using ¹²⁵I-labeled gastrin. We have identified two species of the GR on human stomach and colon cancer cells, as well as normal mouse hepatocytes, with different binding affinities and molecular size. Whether these GR present on GI cancer cells represent one or both of the previously cloned CCK receptors remains unclear.

Studies designed to examine whether GR on cancer cells are functionally linked to the intracellular signal-transduction pathways, and to examine which signal-transduction pathways mediate the growth-regulatory effects of gastrin, have been a major focus of our investigations. We have shown that GR may stimulate any of the effector systems (cAMP, PI hydrolysis and Ca²⁺-calmodulin), depending upon the cell line. Gastrin stimulated the production of cyclic AMP (cAMP) in LoVo, COLO 320, and HCT116 cells, while gastrin stimulated phosphatidylinositol hydrolysis and mobilization of [Ca²⁺]ₗ in HT-29 cells. Gastrin also stimulated increases of [Ca²⁺]ₗ independent of PI hydrolysis in AGS-P human stomach cancer cells. The growth-regulatory effect of gastrin on these cancer cells (stimulatory on LoVo, COLO 320, AGS-P, and HT-29 cells; inhibitory on HCT116 cells) was correlated with the effect of gastrin on the signal-transduction pathway in each cell line. Gastrin thus appears to regulate growth of human GI cancer cells through gastrin receptor-linked signal-transduction pathways that are cell-specific.

The mechanism whereby an increase in cAMP can either stimulate or inhibit growth (growth of LoVo

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**Fig. 1.** Tumor area of two human pancreatic adenocarcinomas (SKI, CAV) and a human cholangiocarcinoma (SLU-132) following 8 weeks of caerulein treatment. *P < 0.05 vs control.