COOH-terminal 26-amino acid residues of progastrin are sufficient for stimulation of mitosis in murine colonic epithelium in vivo

P. D. Ottewell, A. Varro, G. J. Dockray, C. M. Kirton, A. J. M. Watson, T. C. Wang, R. Dimaline, and D. M. Pritchard. COOH-terminal 26-amino acid residues of progastrin are sufficient for stimulation of mitosis in murine colonic epithelium in vivo. Am J Physiol Gastrointest Liver Physiol 288: G541–G549; 2005. First published October 14, 2004; doi:10.1152/ajpgi.00268.2004.—Transgenic mice (hGAS) that overexpress human progastrin are more susceptible than wild-type mice (FVB/N) to the induction of colonic aberrant crypt foci (ACF) and adenomas by the chemical carcinogen azoxymethane. We have previously shown significantly increased levels of colonic mitosis in hGAS compared with FVB/N mice after γ-radiation. To investigate whether the effects of progastrin observed in hGAS colon require the presence of other forms of circulating gastrin, we have crossed hGAS mice with gastrin knockout (G−/−) mice to generate mice that express progastrin and no murine gastrin (G−/−·G−/−). After azoxymethane, G−/−·G−/− mice developed significantly more ACF than control G−/−·G−/+ mice (which do not express any forms of gastrin). G−/−·G−/+ mice also exhibited significantly increased colonic mitosis both before and after exposure to 8 Gray Gy γ-radiation or 50 mg/kg azoxymethane compared with G−/−·G−/−. Treatment of G−/−·G−/+ mice with synthetic progastrin (residues 21–101 of human progastrin) or G17 extended at its COOH terminus corresponding to the COOH-terminal 26-amino-acid residues of human progastrin (residues 76–101, G17-CFP) resulted in continued colonic epithelial mitosis after γ-radiation, whereas glycine-amidated gastrin (G17) that overexpress human amidated gastrin. In this case, the transgene consists of human preprogastrin, which results in transgene expression in the stomach (12), although lower levels of expression are also found in the human duodenum and pituitary (12, 29). Gastrin occurs in various molecular forms. The human COOH-terminally amidated gastrins, G17 and G34, are generated from a 101-amino acid precursor molecule, progastrin, by post-translational modifications (Fig. 1). Preprogastrin is cotranslationally translocated into the endoplasmic reticulum where the signal peptide is rapidly cleaved to give rise to progastrin (38). Other forms of progastrin, such as the COOH-terminal 26-amino acid residues of the peptide used in current studies, have also been identified in vivo (26, 37). Progastrin is subsequently cleaved by prohormone convertases and carboxypeptidase E to give rise to a peptide with a COOH-terminal glycine residue, namely G34-Gly, and an additional cleavage generates the peptide G17-Gly. G34-Gly can be converted to the COOH terminally amidated peptide G34 by peptidyl α-amidating monooxygenase (11) and can similarly be cleaved to generate G17 (Fig. 1). G17 is the predominant antral form of gastrin, with nonamidated precursors (progastrin, glycine-extended gastrin) generally comprising <10% of the total secreted peptide in humans. However, in certain clinical situations where processing is impaired, a greater proportion of nonamidated gastrin is secreted. For example, tissue and plasma concentrations of progastrin are elevated in some patients with colorectal carcinoma (7, 24, 30, 36), and a mixture of gastrin forms may be secreted by gastrinomas (2).

Powerful evidence for the importance of the gastrin family of peptides in the colon has emerged from studies involving transgenic mice. hGAS mice express a human preprogastrin transgene in the liver. Because progastrin cannot be processed by hepatocytes, these mice have high circulating concentrations of progastrin (1–100 nM) and normal plasma concentrations of murine gastrin (40). hGAS mice show increased proliferation and hyperplasia of colonic crypts (40) and are more sensitive than their wild-type counterparts to the induction of aberrant crypt foci (ACF; which are an early histological indicator of colonic tumor development) and colonic adenomas after treatment with the carcinogen azoxymethane (AOM; see Refs. 31 and 32). INS-GAS mice overexpress human amidated gastrin. In this case, the transgene consists of the rat insulin promoter upstream of the coding sequence of human preprogastrin, which results in transgene expression specifically in the β-cells of the pancreatic islets and elevated...
plasma amidated gastrin (~150 pM; see Ref. 40). INS-GAS mice show similar, although less pronounced, increased proliferation and hyperplasia within colonic crypts (40) but do not show increased sensitivity to carcinogen-induced tumors (31, 32). MTI/G-Gly mice carry the mouse metallothionein-human gastrin transgene, resulting in overexpression of G34-Gly. These mice exhibit significantly increased colonic proliferation, increased colonic mucosal thickness, and a 41% increase in goblet cells (18).

Increased sensitivity to AOM-induced colonic carcinogenesis has also been reported in gastrin knockout mice (10). Cobb et al. (10) therefore conclude that normal serum concentration of murine gastrin may protect against colon carcinogenesis (10). Therefore, it is currently unclear whether the presence of amidated gastrin suppresses the procarcinogenic effects of progastrin in murine colonic epithelia.

We have previously reported that, after DNA damage by 8 Gy γ-radiation, colonic mitosis persisted at higher rates in hGAS compared with FVB/N and INS-GAS mice and that this was associated with altered expression of the cell cycle regulatory proteins cdk4 and cyclin D1 (25). To investigate whether the effects observed in hGAS colonic epithelium require the presence of other circulating forms of gastrin, we have crossed with gastrin knockout (G−/−) mice generated through the targeted deletion of the murine gastrin gene, resulting in complete absence of gastrin (17). The resulting progeny (G+/− hGAS) were backcrossed to G−/− mice for five generations. G−/− hG+/+ mice were selected on the basis of absence of amidated gastrin and presence of progastrin by serum RIA. The resulting G−/− hG+/+ mice were crossed, and their progeny were genotyped as described below by real-time PCR to generate colonies of G−/− hG+/+ mice, which express progastrin and no murine gastrin, and G−/− hG−/− mice, which do not express gastrin. G−/− hG+/+ and G−/− hG−/− mice had identical genetic backgrounds, which was predominantly C57BL/6.

All animals were reared in a conventional animal facility. Mice were between the ages of 10 and 12 wk, and 6 male and 6 female mice were used in each experimental group. Mice were fed a commercially prepared pellet diet and allowed water ad libitum. The animals were maintained on a 12:12-h light-dark cycle, and all experiments were conducted during the daytime.

Genotyping. Tail snips from mice were incubated overnight at 55°C in 50 mM Tris·HCl, pH 7.5, 10 mM EDTA, 100 mM NaCl, and 0.5% SDS containing proteinase K (0.75 mg/ml). Digests were centrifuged, and the supernatant was extracted with saturated phenol (pH 8) then phenol chloroform (pH 8). The aqueous phase was precipitated with sodium acetate and ethanol, the pellet was dissolved in 0.25 ml Tris-EDTA, and the concentration was determined by absorbance at 260 nm. The concentration of DNA in each sample was adjusted to 2.5 μg/ml before analysis by real-time PCR, using Taqman chemistry and an ABI Prism 7700 Sequence Detection system (Applied Biosystems, Warrington, UK). The relative abundance of genomic DNA encoding human preprogastrin in the samples was determined using primer sequences 5′-ggacagtagaagctegctg (forward) and 5′-aagagctacctacccagga (reverse) together with the probe 5′-1gctgctagcgtggccaggtt. These primers generate a 105-bp amplicon that includes the last four bases of exon 2 and 101 bases of the small second intron of the human gastrin gene. The relative abundance of genomic DNA encoding GAPDH in the same samples was determined using Taqman GAPDH Control Reagents (Applied Biosystems). Genotypes were assigned by comparing the relative abundance of DNA encoding human preprogastrin and GAPDH in tail DNA samples using the comparative cycle threshold method. Ratios of genomic human preprogastrin-GAPDH in G−/− hG+/+ were two times those in G−/− hG−/− animals. Gastrin peptide and gene sequences were undetectable in G−/− hG−/− animals.

Assessment of apoptosis and mitosis. For studies involving radiation, mice were subjected to 8 Gy γ-irradiation using a 137Cs source at a dose rate of 2.6 Gy/min and killed 4.5 h after exposure. For studies involving AOM, mice were given an intraperitoneal injection of 50 mg/kg AOM (National Cancer Institute, Midwest Research Institute, Kansas City, MO) and killed 24 h after treatment. The kinetics of the development of apoptotic and mitotic cells in the murine intestine after γ-radiation have previously been established (27, 41), and maximal levels of colonic apoptosis are induced 4.5 h after 8 Gy γ-irradiation (27). AOM dose-response and time-course studies were initially performed in wild-type mice. Maximal induction of colonic apoptosis and maximal suppression of colonic mitosis occurred after 24 h. AOM (50 mg/kg) caused greater induction of colonic apoptosis and greater suppression of mitosis than 10 mg/kg without compromising animal health over a 24-h period. To study whether progastrin
overexpression affected colonic mitosis after AOM, we used 50 mg/kg AOM to maximally suppress mitosis. Animals were killed by CO₂ asphyxiation, and their intestines were removed and fixed in Carnoy’s fixative. Transverse sections of the colon (3 µm) were prepared and stained with hematoxylin and eosin. Fifty half-crypts per mouse were scored for apoptosis and mitosis using morphological criteria on a cell positional basis as previously described and validated in detail (20, 21, 28). Data are presented as mean apoptotic/mitotic index percentage for a group of six mice or as plots of apoptotic/mitotic cell index percentage against cell position along the crypt (20, 21, 28).

Bromodeoxyuridine immunohistochemistry. Control and irradiated mice were given an intraperitoneal injection of 80 mg/kg bromodeoxyuridine (BrDU; Sigma, Dorset, UK) 1 h before death. Tissue sections were prepared as described above except that tissues were fixed in 4% formaldehyde in PBS rather than Carnoy’s fixative. BrDU immunohistochemistry was performed as previously described (40). The primary antibody was mouse monoclonal anti-BrDU (DakoCytomation, Cambridge, UK) at a dilution of 1:175, and an anti-mouse biotinylated secondary antibody (DakoCytomation) was used at a dilution of 1:200. Fifty half-crypts per mouse were scored on a cell positional basis according to whether or not cells were BrDU positive.

Induction of ACFs in animals with the chemical carcinogen AOM. Mice were injected intraperitoneally with 10 mg/kg AOM per week for 3 wk; all animals were culled 2 wk after the last injection. This method has previously been shown to be optimal for inducing ACF in colon of hGAS and FVB/N mice (32). Animals were killed by CO₂ asphyxiation; the colon was removed and flushed with saline. The colons were split open by midline incision and separated into three sections of equal length, the most proximal being cecum, the middle section being midcolon, and the most distal being rectum. Sections were pinned flat on paraffin wax and fixed with 4% formal saline for 24 h before being transferred to 70% ethanol. Colon sections were briefly stained with methylene blue (0.3%) for enumeration of ACFs, as previously described (10, 32). Total number of ACFs per section was scored using light microscopy (×20 magnification).

Assessment of the effects of the COOH-terminal flanking peptide of progastrin. To investigate which portions of the progastrin molecule were causing effects on colonic epithelia, glycine-extended gastrin-17 (G17-Gly), human preprogastrin-(76–101) (i.e., G17-CFP, amino acid sequence QGPWLEEEAYGWMDFGR, cross-reacting with antibodies L289 and L2; Multi- peptide systems, San Diego, CA) were used because YGRRSAEDEN was used because of its intermediate length between the other two peptides. The responses to these peptides were compared with the response of G⁻/⁻ hGAS mice to synthetic progastrin [preprogastrin-(21–101)]. This peptide was synthesized in Dr. Timothy Wang’s laboratory on an Applied Biosystems Pioneer peptide synthesizer and, after cleavage, was purified using RP-HPLC. Purity was >90.0%, and the molecular weight estimated by MALDI-TOF DE was 9,084, close to the predicted molecular weight of 9,082. The lyophilized powder was dissolved in water before injection in mice. Each of these peptides (20 nmol) was administered to G⁻/⁻ hGAS mice by intraperitoneal injection 30 min before exposure to 8 Gy γ-radiation. Alternatively, G⁻/⁻ hGAS mice received an intraperitoneal injection of 0.1 ml antibody raised against G17-CFP (L517) or of preimmune serum 30 min before exposure to 8 Gy γ-radiation. L517 was produced by conjugating the G17-CFP to BSA using glutaraldehyde, and the conjugate was used to immunize rabbits according to published protocols (39). L517 has been demonstrated to bind to progastrin and its COOH-terminal fragments but not to amidated or glycine-extended gastrins (data not shown).

Analysis of plasma gastrin and progastrin concentrations.RIA for amidated gastrin was performed as described previously using antibody L2 (40). Plasma concentrations of progastrin were measured using the progastrin specific antibody L289 (40).

Statistical analysis. In experiments where both male and female mice were analyzed, two-way ANOVA was used to assess significant differences between genotypes and sexes as well as any interaction between genotype and sex. In experiments where only male animals were analyzed, a Student’s t-test was used to assess significant differences between genotypes. A modified median test was used to assess significant differences at individual cell positions (25, 28). We have interpreted differences as being significant at P < 0.05 by two-way ANOVA or Student’s t-test, and there are significant differences at more than three consecutive cell positions in the modified median test.

RESULTS

G⁻/⁻ hGAS mice express progastrin but no murine gastrin. The G⁻/⁻ hGAS mice generated by crossing hGAS and gastrin knockout mice were viable and had similar fertility and growth characteristics to G⁻/⁻ hGAS mice. G⁻/⁻ hGAS mice had a mean serum progastrin concentration of 0.99 ± 0.58 nM (n = 48). No statistically significant differences in serum progastrin concentration were observed between control male and female mice, nor was the serum concentration significantly altered by any of the treatments administered (data not shown). No amidated gastrin was detected in G⁻/⁻ hGAS mice. progastrin and amidated gastrin were not detected in G⁻/⁻ hGAS mice.

G⁻/⁻ hGAS mice show increased colonic epithelial mitosis. In the resting state, G⁻/⁻ hGAS mice showed significantly increased rates of colonic mitosis compared with G⁻/⁻ hGAS mice (P < 0.001 by ANOVA and cell positions 3–33 significantly different by modified median test; Fig. 2, A and C, and Fig. 3, B and D). In addition to this assessment of cells in the M phase of the cell cycle, BrDU staining of colonic tissue sections from G⁻/⁻ hGAS mice and G⁻/⁻ hGAS mice was performed to assess the proportion of cells in the S phase of the cell cycle (6 male mice in each experimental group). The BrDU labeling index in untreated male G⁻/⁻ hGAS mice (6.24 ± 1.04%) was significantly higher than male G⁻/⁻ hGAS mice (3.22 ± 1.14%; Fig. 2, B and D, P < 0.001 by t-test and significant differences at cell positions 7–18 by modified median test). There was no significant difference in the baseline apoptotic index of colonic crypts in G⁻/⁻ hGAS and G⁻/⁻ hGAS mice (Fig. 3, A and C). No differences in apoptotic or mitotic indexes were detected between the sexes of either genotype of animals, and no interaction between sex and genotype was observed. Mucosal thickness was increased in G⁻/⁻ hGAS mice (3.22 ± 3.04 cells/hemicrypt) compared with G⁻/⁻ hGAS mice (3.04 cells/hemicrypt) compared with G⁻/⁻ hGAS mice (3.04 cells/hemicrypt). No differences in apoptotic or mitotic indexes were detected between the sexes of either genotype of animals, and no interaction between sex and genotype was observed. Mucosal thickness was increased in G⁻/⁻ hGAS mice (3.22 ± 3.04 cells/hemicrypt) compared with G⁻/⁻ hGAS mice (3.04 ± 3.04 cells/hemicrypt; P < 0.01 by ANOVA with no interaction between sex and genotype) mice.

Colonic mitosis persists after DNA damage by γ-radiation or AOM in G⁻/⁻ hGAS mice. G⁻/⁻ hGAS mice showed persistent mitotic figures in colonic crypts after both 8 Gy γ-radiation and AOM treatment (Fig. 2G). After 8 Gy γ-radiation (4.5 h), the mean colonic mitotic index was significantly lower in G⁻/⁻ hGAS mice compared with G⁻/⁻ hGAS mice (Fig. 4, B and D; ANOVA P < 0.001 and significant differences at cell positions 4–35 by modified median test). No significant differences were observed between male and female mice, and no interaction between sex and genotype was observed. Similarly, the mean colonic mitotic index 24 h after 50 mg/kg AOM treatment was
significantly lower in G<sup>−/−</sup>-hg<sup>−/−</sup> mice compared G<sup>−/−</sup>-hg<sup>+/+</sup> mice (Fig. 5, B and D; ANOVA P < 0.001 and significant differences at cell positions 5–19 by modified median test). No significant differences were seen between male and female mice, and no interaction between sex and genotype was observed. The cell positional distribution of mitotic cells did not alter after either radiation or AOM treatment of G<sup>−/−</sup>-hg<sup>+/+</sup> mice, and proliferating cells were present along the entire length of the colonic crypt (Figs. 4D and 5D).

G<sup>−/−</sup>-hg<sup>+/+</sup> mice also showed an increased BrdU labeling index 4.5 h after exposure to 8 Gy γ-radiation. The BrdU labeling index of male G<sup>−/−</sup>-hg<sup>+/+</sup> mice was 4.99 ± 0.58% compared with 1.35 ± 0.38% in male G<sup>−/−</sup>-hg<sup>−/−</sup> mice. This difference was significant by Student’s t-test (P < 0.001) and by modified median test (significant cell positions 7–25; Fig. 2, F and H).

Apoptotic bodies were observed at the base of colonic crypts from both G<sup>−/−</sup>-hg<sup>+/+</sup> and G<sup>−/−</sup>-hg<sup>−/−</sup> mice 4.5 h after 8 Gy γ-radiation (Fig. 2, E and G, and Fig. 4, A and C) and 24 h after AOM (Fig. 5, A and C). No significant differences in colonic apoptotic indexes were seen 4.5 h after 8 Gy γ-radiation. However, 24 h after 50 mg/kg AOM, G<sup>−/−</sup>-hg<sup>+/+</sup> mice showed significantly less colonic apoptosis compared with G<sup>−/−</sup>-hg<sup>−/−</sup> mice (ANOVA P < 0.05). This was largely because female G<sup>−/−</sup>-hg<sup>+/+</sup> mice exhibited significantly less colonic apoptosis than male G<sup>−/−</sup>-hg<sup>+/+</sup> mice (ANOVA P < 0.05), with a positive interaction between sex and genotype of P < 0.05 as assessed by two-way ANOVA. Differences in apoptotic indexes were, however, found not to be significant at any cell position along the colonic crypt when assessed by the modified median test.

G<sup>−/−</sup>-hg<sup>+/+</sup> mice are more susceptible to colonic carcinogenesis. Five weeks after three weekly injections of 10 mg/kg AOM, G<sup>−/−</sup>-hg<sup>+/+</sup> mice developed significantly more ACF per colon compared with G<sup>−/−</sup>-hg<sup>−/−</sup> mice (Fig. 6). This was statistically significant by two-way ANOVA (P < 0.01), with no significant differences observed between male and female mice and no interaction between sex and genotype. The majority of ACF developed in the midcolon (G<sup>−/−</sup>-hg<sup>+/+</sup>; male = 3.5 ± 1.64, female = 6.0 ± 4.8; G<sup>−/−</sup>-hg<sup>−/−</sup>; male = 2.14 ± 1.57, female = 4.06 ± 2.04) and rectum (G<sup>−/−</sup>-hg<sup>+/+</sup>; male = 5.5 ± 2.16, female = 4.8 ± 1.3; G<sup>−/−</sup>-hg<sup>−/−</sup>; male = 2.57 ± 1.27, female = 2.2 ± 2.94), with the fewest being observed in the cecum (G<sup>−/−</sup>-hg<sup>+/+</sup>; male = 2.8 ± 1.7, female = 2.4 ± 1.14; G<sup>−/−</sup>-hg<sup>−/−</sup>; male = 1.4 ± 0.97, female = 1.2 ± 1.36; Fig. 6). In both G<sup>−/−</sup>-hg<sup>+/+</sup> and G<sup>−/−</sup>-hg<sup>−/−</sup> ~80% of the total ACFs was singlets and 20% was doublets or multiplets (data not shown).

Human preprogastrin-(76–101) (G17-CFP) causes increased colonic mitosis. To determine which portion of the preprogastrin molecule was responsible for the biological effects described above, G<sup>−/−</sup>-hg<sup>−/−</sup> mice were injected with synthetic human preprogastrin-(21–101) (progastrin), human preprogastrin-(76–101) (G17-CFP), G17-Gly, human preprogastrin-(96–101) (SAEDEN), or human preprogastrin (Y<sup>−/−</sup>)-(93–101) (YGRRSAEDEN) (Fig. 1) before exposure to γ-radiation, and colonic mitosis was assessed on a cell positional basis. Pretreatment of G<sup>−/−</sup>-hg<sup>−/−</sup> mice with progastrin or G17-CFP resulted in continued colonic mitosis 4.5 h after γ-radiation. After pretreatment with progastrin or G17-CFP, the colonic mitotic index of G<sup>−/−</sup>-hg<sup>−/−</sup> mice was significantly increased.
compared with saline-treated animals [Fig. 7B; \( P < 0.001 \) for each peptide by Student’s \( t \)-test and significant differences at cell positions 5–20 (progastrin) and 5–23 (G17-CFP) by modified median test]. Pretreatment with G17-Gly, SAEDEN, or YGRRSAEDEN showed no effect on colonic mitosis of \( G^{-/-} \) mice 4.5 h after exposure to 8 Gy \( \gamma \)-radiation (Fig. 7B).

To confirm the idea that the COOH-terminal 26-amino acid residues of progastrin (G17-CFP) were responsible for the
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Fig. 5. Colonic apoptotic and mitotic index from G−/− hg+/+ and G−/− hg−/− mice 24 h after 50 mg/kg azoxymethane (AOM). Six mice were analyzed in each experimental group. A and B: mean ± SE apoptotic (A) and mitotic (B) index (%). C and D: cell positional distribution of apoptosis (C) and mitosis (D). *Significant differences between sexes by 2-way ANOVA (P < 0.05). • Significant differences between mouse genotypes by 2-way ANOVA (P < 0.05) and modified median test (>3 consecutive cell positions).

Fig. 6. Mean ± SE no. of aberrant crypt foci per colon from G−/− hg+/+ and G−/− hg−/− mice 2 wk after 3 × weekly injections of 10 mg/kg AOM treatment (6 mice/experimental group). ACF, aberrant crypt foci. There were no significant differences between sexes. *Significant differences between mouse genotypes by 2-way ANOVA.

effects described above, G−/− hg+/+ mice were treated with an antibody to G17-CFP (L517), or preimmune serum, before exposure to 8 Gy γ-radiation. Colonic mitosis was again assessed on a cell positional basis. Immunoneutralization of G−/− hg+/+ mice decreased colonic mitosis 4.5 h after 8 Gy γ-radiation compared with mice treated with preimmune serum (Fig. 7A). This was highly significant by Student’s t-test (P < 0.001) and by modified median test (significant at cell positions 5–25).

DISCUSSION

There is emerging evidence that amidated and nonamidated gastrins require each other’s presence to exert certain biological effects. For example, glycine-extended gastrin and gastrin 17 synergize to increase gastric acid secretion in gastrin knockout mice (6). However, it has not previously been investigated whether progastrin requires the presence of other circulating forms of gastrin to cause effects on colonic epithelia in vivo. In the present study, no significant differences were observed between male and female mice. A systematic analysis was performed, since female gastrin knockout mice have...
The proliferative effects of progastrin observed in the colonic epithelium of G−/−hg+/+ animals were not observed in small intestinal epithelia (data not shown). We have previously reported no significant differences in small intestinal mitosis in hGAS mice compared with their wild-type counterparts (25). Previous studies have indicated that members of the gastrin family are able to exert effects on the small intestinal epithelium. For example, transgenic overexpression of glycine-extended gastrin (MT1/G-Gly; see Ref. 18) and rats infused with glycine-extended gastrin (1) both exhibit colonic hyperplasia. However, administration of glycine-extended gastrin-17 did not prevent the suppression of colonic mitosis observed in G−/−hg−/− mice after γ-radiation. Therefore, it seems that G17-Gly and G17-CFP may be exerting their effects on the colon in different ways. One possibility is that G17-Gly and G17-CFP act via different receptors. Precursor forms of gastrin, such as progastrin and glycine-extended gastrin, show an ~1,000-fold lower affinity for the gastrin/CK8 receptor than does amidated gastrin (35). Although the existence of a specific receptor for Gly-gastrin has been suggested by radioligand binding studies (35), our data suggest that this is distinct from the receptor for progastrin. Our data suggest that the putative progastrin receptor may be expressed by proliferating cells within colonic crypts. These
findings may have implications for colonic carcinogenesis and for future development of therapies against colon cancer.

The gastrin gene is expressed in many human colon cancer cell lines and human colon carcinomas (13, 24). During colonic carcinogenesis, mutations occur in oncogenic ras and the β-catenin/T cell factor 4 pathways, both of which have been shown to increase gastrin gene expression (16, 23). In addition, the posttranslational processing of progastrin is often incomplete in colon cancer, resulting in increased expression of precursor forms of gastrin (3, 7, 15, 24, 30, 36). Gastrin/CKβB receptor mRNA has been detected by RT-PCR in a proportion of human colorectal cancers (5, 8, 22, 42) and also in some specimens of normal mouse (19) and human (5) colon. However, in the mouse, gastrin/CKβB transcripts were not detected by Northern blot analysis, suggesting that mRNA is present at low abundance. The cell types expressing the gastrin/CKβB receptor are not currently known. Several investigators have therefore proposed that the progastrin and glycine-extended gastrin produced by colorectal neoplasms act in an autocrine or paracrine fashion to stimulate cell growth and hence expand the number of transformed cells (14, 33). Studies involving administration of carcinogens to transgenic mice have additionally provided strong evidence that progastrin has procarcinogenic properties (9, 31, 32). However, the hGAS and Fabp-PG mice previously used in these studies both express normal circulating levels of murine gastrin. Our current studies confirm the observations previously made in hGAS mice that progastrin increases proliferation and carcinogenesis in murine colonic epithelia (9, 25, 31, 32). In addition, they suggest that progastrin increases proliferation and carcinogenesis in murine colonic epithelia (9, 25, 31, 32). In addition, they propose that the progastrin and glycine-extended gastrin acts as an autocrine growth factor in a nontransformed colon cell line. Gastroenterology 113: 1576 –1588, 1997.

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