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Oncogenic ras induces gastrin/CCKB receptor gene expression in human colon cancer cell lines LoVo and Colo320HSR

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Gastrin has the ability to stimulate cell growth in some colorectal cancer cells and some of these cells also express gastrin/CCKB receptors, suggesting that gastrin and its autocrine loop are involved in their proliferation. We previously reported that oncogenic ras induced gastrin gene expression in colon cancer cells. The aim of this study was to investigate whether oncogenic ras also induces gastrin/CCKB receptor gene expression. A transiently transfected activated ras vector stimulated gastrin/CCKB receptor transcriptional activities in both Colo320HSR and LoVo cells, but these ras-increased activities were inhibited by a specific MEK inhibitor, PD98059. An RPA demonstrated that activated ras increased endogenous gastrin/CCKB receptor mRNA levels and PD98059 decreased them in LoVo cells. These findings suggest that oncogenic ras induces gastrin/CCKB receptor gene expression through some intracellular signaling pathways, including MEK, in colon cancer cell lines. (J Lab Clin Med 2003;141:335-41)

Abbreviations: APC = adenomatous polyposis coli; bp = base pair; CCKB = cholecystokinin B; DMSO = dimethylsulfoxide; EDTA = ethylenediaminetetraacetic acid; ERK = extracellular signal-regulated kinase; FCS = fetal calf serum; GAPDH = glyceraldehyde phosphate dehydrogenase; gly-gastrin = glycine-extended gastrin; MAPK = mitogen-activated protein kinase; MEK = MAPK/ERK kinase; mRNA = messenger RNA; RLU = relative light unit; RPA = ribonuclease-protection assay; RT-PCR = reverse transcription-polymerase chain reaction; SDS = sodium dodecyl sulfate; SEM = standard error of the mean; TCF-4 = T-cell factor-4

Gastrin, a peptide hormone that mainly stimulates gastric-acid secretion by parietal cells in the stomach, has been recognized as a trophic factor in colorectal cancer cells,[1] as well as in some other malignant tumor cells.[2-6] However, circulating gastrin is probably not involved in colorectal tumorigenesis; patients with Zollinger-Ellison syndrome, in whom extreme hypergastrinemia develops, do not have an increased risk of colon cancer.[7] The authors of a recent case-control study reported that high serum gastrin levels could account for 8.6% of colorectal cancer cases[8]; it is therefore possible that gastrin promotes the progress of colorectal cancer in another manner.

Gastrin/CCKB receptor is a member of the superfamily of seven-transmembrane guanine nucleotide-binding protein (G protein)–coupled receptors.[9] It is mainly expressed in the central nervous system and stomach but not, however, in normal colonic mucosa. We previously reported that gastrin/CCKB receptor was expressed in some colon cancer cells, including LoVo.[10]

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Moreover, a recent study involving a RT-PCR method demonstrated that gastrin and the gastrin/CCKB receptor were expressed in 78% and 81% of colonic adenomatous polyps, respectively, and the receptor was coexpressed with gastrin in 97% of the polyps that expressed the gastrin/CCKB receptor gene.[11] These results suggest that gastrin enhances colorectal cancer cell proliferation in an autocrine manner by way of the gastrin/CCKB receptor, as noted in another report.[12]

The cellular protooncogene ras has a critical role in transducing extracellular signals for growth and differentiation[13] and, when activated, will initiate protein kinase cascades that ultimately have an effect on the transcription factors governing gene expression. On the basis of the finding that ras-activated mutations are highly prevalent in colon cancer,[14] we demonstrated that oncogenic ras induces gastrin gene expression through activation of the Ras-Raf-MEK-ERK signal-transduction pathway.[15]

If gastrin is induced by oncogenic ras and promotes cell proliferation in an autocrine manner in colon cancer cells, it stands to reason that oncogenic ras also induces gastrin/CCKB receptor gene expression. Although many researchers have demonstrated the involvement of the gastrin/CCKB receptor in colorectal tumorigenesis or cell proliferation, few have investigated how it is involved in colorectal cancers. In this study, we analyzed the effect of oncogenic ras on gastrin/CCKB receptor gene expression in two human colon cancer cell lines, Colo320HSR and LoVo, so that we might examine our hypothesis that oncogenic ras induces gastrin/CCKB receptor gene expression in these colon cancer cells.

METHODS

Cell lines. Colo320HSR and LoVo were purchased from Dainippon Pharmaceutical Co (Osaka, Japan). Cells were cultured in RPMI-1640 (Colo320HSR) or Ham's F12 (LoVo) medium, each supplemented with 10% FCS (ICN, Aurora, Ohio) and 1% penicillin (5000 U/mL)/streptomycin (5000 mg/mL) (Gibco-BRL, Gaithersburg, Md) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Nested RT-PCR. Colo320HSR or LoVo cells were plated 2.0×10^6 cells/dish and cultured for 48 hours. Total RNA was extracted from the cells with an RNAqueous kit (Ambion, Austin, Texas). Total RNA was also extracted with the same method from a human fundic gastric mucosa biopsy sample. Five micrograms of total RNA was reverse-transcribed into complementary DNA with the Superscript Preamplication System (Gibco-BRL) and then amplified 35 times with human gastrin/CCKB receptor-specific primers: 5'-TTGGAGCTGGCCATTAG-3' (sense) and 5'-CACTGTCGCCGTCAAAG-3' (antisense) for the first-round PCR and 5'-ATGCTCATCATCGTGGTC-3' (sense) and 5'-AGAGATAAGCCCGTAGGC-3' (antisense) for the second-round PCR. Denaturation was set at 94°C, annealing at 46°C for the first round and 50°C for the second round, and extension at 72°C. Each PCR product was analyzed on 1.0% agarose gel electrophoresis.

Transfection and transcriptional analysis. Colo320HSR cells and LoVo cells were each plated 2.0×10^5 cells/well in six-well plates (Falcon; Becton-Dickinson, Lincoln Park, NJ) and cultured for 24 hours. Cells were cotransfected with reporter GaR-Luc and ras expressing pCMV-RasV12 or pCMV-Ras (Clontech, Palo Alto, Calif) with internal transfection standard pRL-TK (Promega, Madison, Wis) by a cationic liposome–mediated transfection method employing Dosper liposomal transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany) and then incubated for 24 hours. GaR-Luc, a gastrin/CCKB receptor plasmid, is a luciferase reporter containing 514 bp of the 5'-flanking promoter sequence of the human gastrin/CCKB receptor gene (from −533 to −20) that was amplified on PCR from LoVo cell gDNA and subcloned into a pGL3 basic vector (Promega). The expression plasmid pCMV-Ras constitutively expresses the wild-type Ha-ras protein, whereas pCMV-RasV12 expresses a constitutively active form of the Ha-Ras protein that contains a glycine-to-valine mutation at residue 12. An internal transfection standard, pRL-TK, which expresses *Renilla* luciferase, was used to normalize transfection efficiency. Luciferase activities were determined with the Dual-Luciferase Reporter Assay System (Promega) and a MiniLumat LB9506 (Belthold GmbH & Co, Bad Wildbad, Germany). The medium was exchanged for the same base medium, supplemented with 0.5% FCS and 100 μmol/L PD98059 (Calbiochem) in 0.5% DMSO (Wako Pure Chemical, Osaka, Japan) or 0.5% FCS and diluent alone. Cells were harvested for luciferase assay after 30 hours. Cells appeared to tolerate PD98059 treatment well; no significant differences were detected in cell number or structure between the experimental and control cells (diluent treatment) at the time of harvest. LoVo cells were cotransfected with GaR-Luc and pRL-TK, then incubated with various concentrations of PD98059 (0–100 μmol/L) for 30 hours. Cells were also harvested for luciferase assay. Typically, 0.6 μg of the reporter plasmid, 0.6 μg of the expression plasmid, and 6 ng of the internal control plasmid were transfected in each well for Colo320HSR cells, and 1.5 μg of the reporter plasmid, 1.5 μg of the expression plasmid, and 15 ng of the internal control plasmid were transfected for LoVo cells. Data were derived from the mean of triplicate-transfected wells; transfection experiments were repeated three times.

RPA. LoVo cells were plated 2.0×10^6 cells/dish and cultured for 24 hours. The medium was exchanged for the same base medium with 0.5% FCS, with or without 100 μmol/L of PD98059, and the cells were incubated for an additional 30 hours. Total RNA was extracted from the cells as described above. In addition, LoVo cells were transfected with pCMV-Ras or pCMV-RasV12 and incubated for 48 hours, after which total RNA was extracted as described above. Riboprobes were generated from sense templates from human gastrin/CCKB receptor exon 2 (388 nucleotides digested to 249 nucleotides) and human GAPDH (360 nucleo-

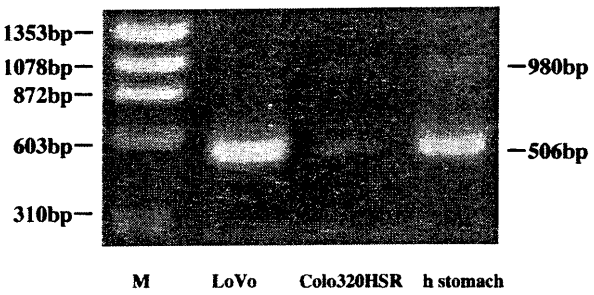

Fig 1. Expression of human gastrin/CCKB receptor in Colo320HSR, LoVo, and human stomach cells. Five micrograms each of total RNA from Colo320HSR, LoVo, and a human fundic gastric mucosa biopsy sample were reverse-transcribed into complementary DNA and amplified consecutively with two sets of human gastrin/CCKB receptor-specific primers. Each PCR product was analyzed on 1.0% agarose gel electrophoresis. *M*, ΦX174/HaeIII size marker.

tides digested to 250 nucleotides). Riboprobes were prepared by means of in vitro transcription (Riboprobe System; Promega) incorporating [α^{32}P]cytidine triphosphate (3000 Ci/mmol; Amersham Pharmacia Biotech, Buckinghamshire, UK). Probes were isolated after urea gel electrophoresis and extraction (shaking in 300 mmol/L sodium acetate, 0.1 mmol/L EDTA, and 0.2% SDS at 50°C for 1 hour). Gastrin/CCKB receptor (5×10^6 cpm) and GAPDH (5×10^6 cpm) riboprobes were combined with 50 μg (for gastrin receptor) and 10 μg (for GAPDH) of total RNA for each reaction, respectively, and hybridized for 16 hours at 57°C (1 mmol/L EDTA;10 mmol/L piperazine-*N,N'-bis*[2-ethanesulfonic acid], pH 6.4; 400 mmol/L NaCl; and 80% formamide). After hybridization, samples were digested with ribonuclease A (20 μg/mL) and ribonuclease T1 (250 U/mL) in 300 mmol/L NaCl, 10 mmol/L Tris (pH 7.4), and 5 mmol/L EDTA at 37°C for 30 minutes. After digestion was stopped, RNA was precipitated and resolved by means of denaturing gel electrophoresis. Density was determined with the use of digitally scanned radiographs, and the results were normalized to GAPDH levels.

Statistical analysis. Results are expressed as mean ± SEM. Student's *t* test for random differences was applied. We considered *P* values of less than .05 statistically significant.

RESULTS

Detection of gastrin/CCKB receptor gene expression. Nested RT-PCR successfully amplified the specific sequence of the human gastrin/CCKB receptor gene, as shown in the 506-bp bands displayed in Fig 1, from total RNA extracted from LoVo, Colo320HSR, and human stomach cells. The 980-bp bands for ColoHSR and human stomach cells were consistent with the amplified sequence from genomic DNA.

Effect of oncogenic ras and MEK inhibitor PD98059 on gastrin/CCKB receptor gene transcriptional activity. Transient transfection experiments with Colo320HSR cells, which demostrate very slight gas-

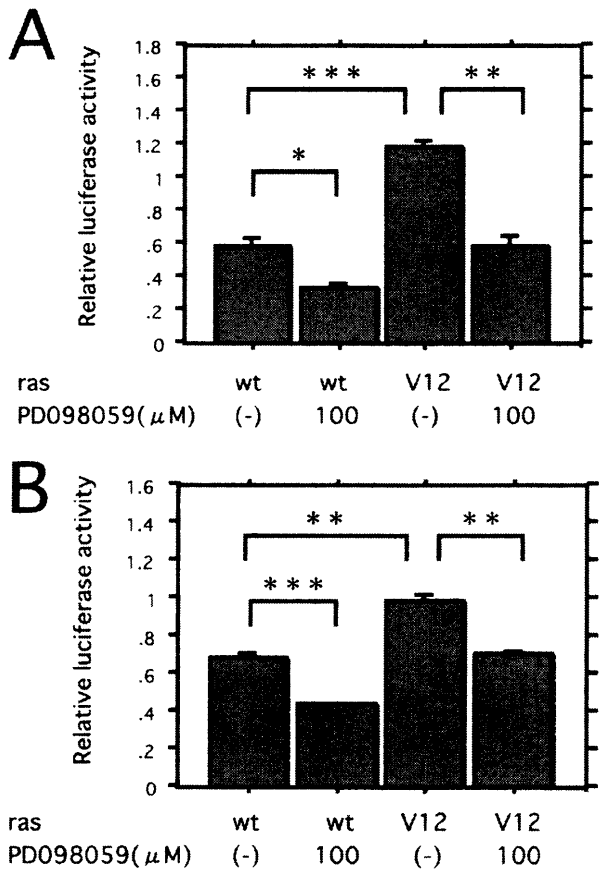

Fig 2. Oncogenic ras activation of gastrin/CCKB receptor–luciferase transcription in (**A**) Colo320HSR cells and (**B**) LoVo cells. Cells were transfected with expression vector pCMV-RasV12 (V12) or pCMV-Ras (wild-type); a reporter plasmid, GaR-Luc; and an internal control plasmid, pRL-TK, by means of a cationic liposome–mediated transfection method. Medium was changed to the same base medium, supplemented in some cases with 100 μmol/L of PD98059 after 24 hours. Luciferase activity was determined after 30 hours of incubation and then normalized to *Renilla* luciferase activity. The relative luciferase activity was the rate of normalized RLUs between each sample and the control sample. Each data point represents the mean of triplicate determinations (±SEM).
$^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

trin/CCKB receptor expression, were performed to directly examine the transcriptional effects of oncogenic ras on gastrin/CCKB receptor-promoter (Fig 2, *A*). Colo320HSR cells were cotransfected with GaR-Luc, pCMV-RasV12, or pCMV-Ras, as well as pRL-TK. Transfection of pCMV-RasV12 stimulated the expression of the cotransfected gastrin/CCKB receptor-promoter–luciferase reporter gene GaR-Luc approximately twofold compared with pCMV-Ras, suggesting up-regulated gastrin/CCKB receptor transcriptional activity by oncogenic ras. A specific MEK inhibitor, PD98059, inhibited the transcriptional activity stimulated by oncogenic ras. Transfection of LoVo cells with pCMV-

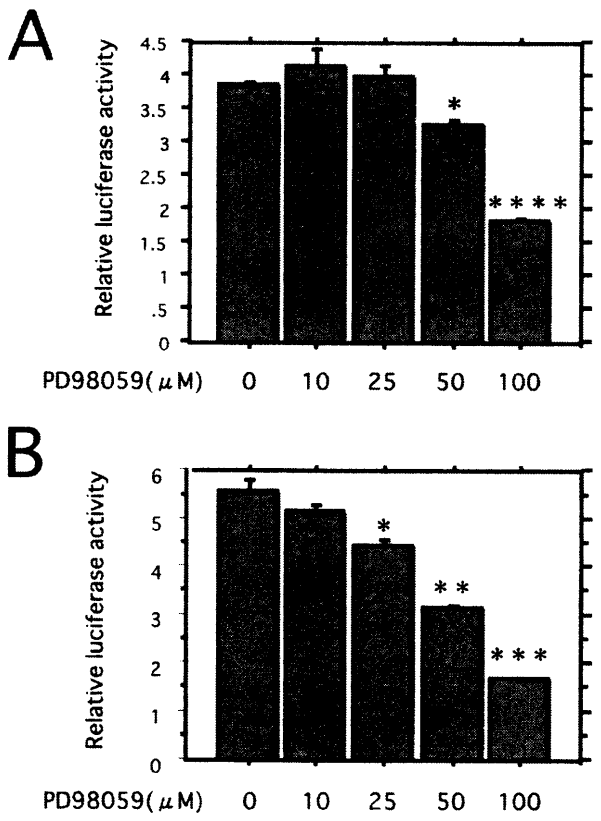

Fig 3. Suppressive effect of PD98059 on gastrin/CCKB receptor–luciferase transcription in LoVo cells. Cells were transfected with a reporter plasmid, GaR-Luc (3 μg/well), and an internal control plasmid, pRL-TK (30 ng/well), by means of a cationic liposome–mediated transfection method. Medium was changed to the same base medium, supplemented with PD98059 (0–100 μmol/L) after 24 hours. Luciferase activity was determined after (**A**) 10 and (**B**) 30 hours of incubation and then normalized to *Renilla* luciferase activity. The relative luciferase activity was the rate of normalized RLUs between each sample and the control sample. Each data point represents the mean of triplicate determinations (±SEM).
$^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$.

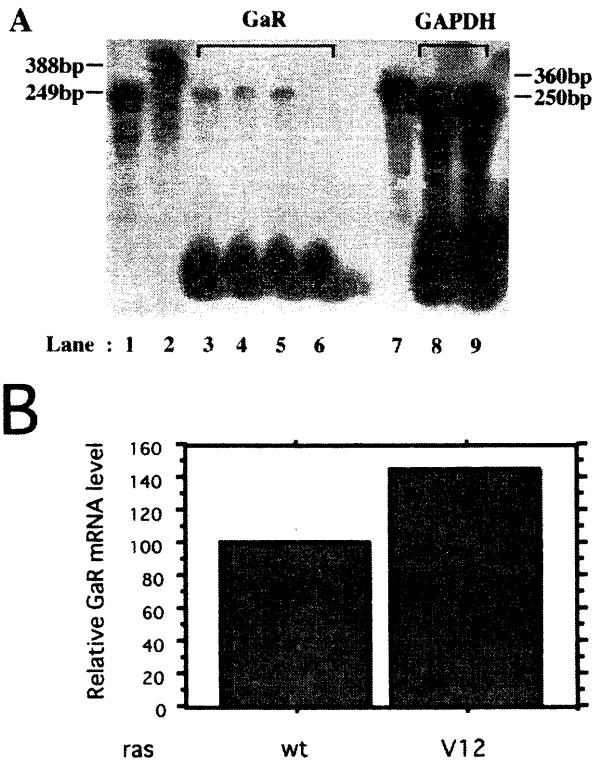

Fig 4. Oncogenic ras activation of gastrin/CCKB receptor mRNA in LoVo cells. **A**, RPA for gastrin/CCKB receptor (*GaR*) and GAPDH message. LoVo cells were transfected with expression vector pCMV-Ras (wild-type) or pCMV-RasV12 (V12). Total RNA was extracted after 48 hours and subjected to RPA as described in the Methods. Digested gastrin/CCKB receptor riboprobes (249 bp) are shown in *lane 4* (pCMV-Ras) and *lane 5* (pCMV-RasV12), and digested GAPDH riboprobes (250 bp) are shown in *lane 8* (pCMV-Ras) and *lane 9* (pCMV-RasV12). The size marker of 231 bp is shown in *lane 1*. Undigested gastrin/CCKB receptor probes (388 bp) are shown in *lane 2*, and the undigested GAPDH probes (360 bp) are shown in *lane 7*. The positive control for gastrin/CCKB receptor message is shown in *lane 3*, and the negative control with transfer RNA is shown in *lane 6*. **B**, Gastrin/CCKB receptor mRNA levels relative to those of normalized GAPDH mRNA. Gastrin/CCKB receptor and GAPDH mRNA levels from RPA (**A**) were measured with the use of densitometry. A shorter radiographic exposure was used for GAPDH-signal determination.

RasV12 stimulated the expression of cotransfected GaR-Luc approximately 1.4-fold compared with pCMV-Ras (Fig 2, *B*). This stimulation of transcriptional activity by oncogenic ras was also inhibited by PD98059.

To investigate the dose effect of PD98059 on the transcriptional activity of gastrin/CCKB receptor-promoter, we transfected LoVo cells with GaR-Luc and treated them with various concentrations of PD98059 (0–100 μmol/L). Luciferase activity was determined after 10 hours (Fig 3, *A*) and 30 hours (Fig 3, *B*) of PD98059 treatment. PD98059 reduced gastrin/CCKB receptor-promoter transcriptional activity after 10 and 30 hours in a dose-dependent manner.

Effect of oncogenic ras expression or PD98059 on endogenous gastrin/CCKB receptor mRNA. An RPA assay, in which endogenous gastrin/CCKB receptor gene expression in LoVo cells was examined, showed that oncogenic RasV12 up-regulated the gastrin/CCKB receptor mRNA level compared with the control (Fig 4, *A*). Furthermore, the RPA density with results normalized to GAPDH levels demonstrated that RasV12 increased gastrin/CCKB receptor mRNA level approximately 1.45 times greater than that of control Ras (Fig 4, *B*), whereas other RPA results showed that PD98059 down-regulated the level of gastrin/CCKB receptor

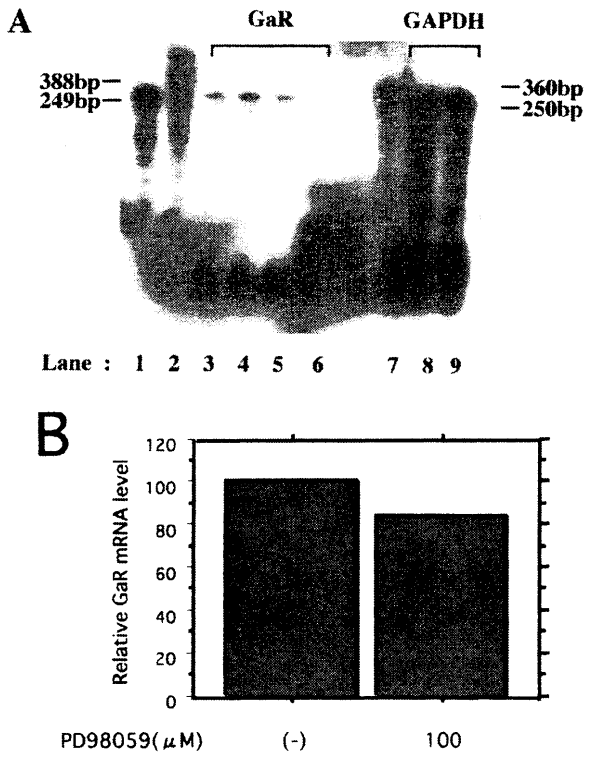

Fig 5. Suppressive effect of PD98059 on gastrin/CCKB receptor mRNA in LoVo cells. **A**, RPA for gastrin/CCKB receptor (*GaR*) and GAPDH message. LoVo cells were treated for 30 hours with or without PD98059 and subjected to RPA as described in the Methods. Digested gastrin/CCKB receptor riboprobes (249 bp) are shown in *lane 4* (without PD98059) and *lane 5* (with PD98059); digested GAPDH riboprobes (250 bp) are shown in *lane 8* (without PD98059) and *lane 9* (with PD98059). The size marker of 231 bp is shown in *lane 1*. Undigested gastrin receptor probes (388 bp) are shown in *lane 2*, undigested GAPDH probes (360 bp) in *lane 7*. The positive control for gastrin/CCKB receptor message is shown in *lane 3*, and the negative control with transfer RNA is shown in *lane 6*. **B**, Gastrin/CCKB receptor mRNA levels relative to those of normalized GAPDH mRNA. Gastrin/CCKB receptor and GAPDH mRNA levels from RPA (**A**) were measured with the use of densitometry. A shorter radiographic exposure was used for GAPDH-signal determination.

mRNA compared with the control (Fig 5, *A*). The RPA density with results normalized to GAPDH levels demonstrated that PD98059 decreased the level of gastrin/CCKB receptor mRNA approximately 0.84 times lower than that of nontreated control cells (Fig 5, *B*). However, an RPA assay with Colo320HSR cells failed to demonstrate the effect of oncogenic ras on gastrin/CCKB receptor mRNA expression; gastrin/CCKB receptor mRNA is extremely low in Colo320HSR cells (data not shown).

DISCUSSION

Several studies have revealed that gastrin has a trophic effect on colorectal cancer cell proliferation. Ishizuka et al[1] reported that gastrin-17 induced cell proliferation in some colon cancer cell lines, including LoVo and Colo320, through the gastrin/CCKB receptor and its intracellular signal-transduction pathways, which were specific in each cell line. Moreover, Smith and Watson[11] demonstrated that gastrin and gastrin/CCKB receptor were expressed in almost all colonic polyps they examined, suggesting that their early activation in the adenoma-carcinoma sequence promoted the progression of colon cancer. Because colon cancers have heterogenous presentations with multifactorial origins, it is reasonable to conclude that colon polyps express the gastrin/CCKB receptor more frequently than do colon cancers.

Fearon and Vogelstein[14] introduced a model for the genetic pathway in the development of colorectal cancer and speculated that a series of gene mutations, including APC tumor suppressor gene and ras oncogene, occur in the progression from normal cells to colorectal cancer. Furthermore, Koh et al[16] demonstrated that gastrin is a target of the β-catenin/TCF-4 growth-signaling pathway in $APC^{min-/+}$ mice intestinal polyps and suggested that activation of gastrin by β-catenin represents an early event in colorectal tumorigenesis and neoplastic progression. In contrast, we suggested that oncogenic ras promotes gastrin gene transcription to cause a proliferation of colorectal cancer cells.[15] Koh et al concluded that gastrin is not a necessary mediator of ras-induced polyp growth, noting that the mutation rate of the ras gene was not significantly different in large polyps between gastrin-deficient and control mice. They only analyzed the codon 12 mutation of the ras gene. However, gene mutations responsible for promoting cell proliferation occur in codons 13 and 61, as well as codon 12, and we found mutations of the K-ras gene in codons 13 and 61 as frequently as we did in codon 12 in human colorectal cancer cells.[15] It therefore seems inappropriate to conclude that gastrin is not a necessary mediator of ras-induced polyp growth. Further investigation is needed, but it is highly possible that gastrin induced by β-catenin as well as that induced by oncogenic ras is involved in colorectal tumorigenesis.

Recent studies have demonstrated that progastrin and gly-gastrin, as well as amidated fully processed gastrin, cause proliferation of colorectal cancer cells[17–19]; novel receptors to which progastrin and gly-gastrin bind specifically have been reported to exist on colon cancer cells but have not been extensively characterized.[17,20,21] In contrast, gastrin/CCKB receptors are reported to be expressed on colorectal cancer cells[10] and to be involved in cell proliferation. Moreover, some gastrin/CCKB receptor antagonists have been shown to suppress colorectal cancer cell proliferation in vivo and

in vitro,[22–24] and an antiserum against the gastrin/CCKB receptor has been shown to inhibit liver invasion by a human colon tumor.[25] These results support the hypothesis that gastrin/CCKB receptor is involved in the proliferation and progression of colorectal cancers.

In this study, we analyzed the effect of oncogenic ras on the expression of gastrin/CCKB receptor gene, speculating that the gastrin/CCKB receptor is located in the autocrine loop if gastrin is involved in the proliferation of colorectal cancer cells. The human colon cancer cell lines examined — LoVo and Colo320HSR — both express the gastrin/CCKB receptor gene. LoVo expresses the gastrin gene and has an oncogenic mutation in the ras gene (K-ras), whereas Colo320HSR does not express the gastrin gene but has the wild-type ras gene. Gastrin/CCKB receptor gene expression was demonstrated on nested RT-PCR analysis (Fig 1). LoVo cells demostrate a higher level of gastrin/CCKB receptor gene expression than Colo320HSR cells, and therefore, because our previous experiments demonstrated that LoVo has the K-ras gene mutation in codon 13 and Colo320HSR does not,[15] the finding of greater gastrin/CCKB receptor gene expression in LoVo cells was consistent with our hypothesis that oncogenic ras increases gastrin/CCKB receptor gene expression. Transfection of pCMV-RasV12 stimulated the luciferase activity of GaR-Luc in Colo320HSR and LoVo cells (Fig 2), suggesting that oncogenic ras expression stimulated gastrin/CCKB receptor transcriptional activity in both cells. We considered the effect of oncogenic ras more prominent in Colo320HSR than in LoVo because the former possessed the wild type K-ras gene. If activation of the ras signal-transduction pathway is in part responsible for induction of gastrin/CCKB receptor transcriptional activity, inhibition of this pathway likely results in decreased activity. The increased effect of oncogenic ras expression on gastrin/CCKB receptor transcriptional activity was partially blocked by PD98059, a specific MEK inhibitor (Fig 2), suggesting that the intracellular signal-transduction pathway that stimulates gastrin/CCKB receptor transcriptional activity includes MEK (eg, the Ras-Raf-MEK-ERK pathway). The inhibition rate of PD98059 on the transcriptional activity of the gastrin/CCKB receptor gene was approximately 42% in both pCMV-ras–transfected cells and pCMV-rasV12–transfected cells in Colo320HSR, which have the wild-type K-ras (Fig 2, *A*), whereas the rate was about 36% in pCMV-ras–transfected cells and 27% in pCMV-rasV12–transfected cells in LoVo, which express oncogenic K-ras (Fig 2, *B*). The abundant ras signaling from constitutively activated K-ras in LoVo cells may cause a lower level of efficacy of PD98059 on the transcriptional activity of the gastrin/CCKB receptor gene. We determined that PD98059 had enough of an effect on pCMV-rasV12–transfected Colo320HSR cells to suppress their transcriptional activity of the gastrin/CCKB receptor gene to a level similar to that seen with pCMV-ras–transfected Colo320HSR cells; Colo320HSR cells have lower levels of ras signaling from wild-type K-ras than LoVo cells, which express oncogenic K-ras. We also found increased expression of gastrin/CCKB receptor mRNA by oncogenic ras demonstrated in LoVo RPA; the specific MEK inhibitor, PD98059, suppressed the mRNA level of gastrin/CCKB receptor (Figs 4 and 5). These results suggest that endogenous gastrin/CCKB receptor mRNA expression was up-regulated by oncogenic ras signaling by way of a pathway that includes MEK (eg, the Ras-Raf-MEK-ERK pathway). It should be considered that oncogenic ras, which is often observed in colorectal cancer cells, may enhance gastrin/CCKB receptor gene expression, as well as gastrin gene expression, and may also accelerate cell proliferation by gastrin in an autocrine manner in human colorectal cancer cells.

We demonstrated enhanced gastrin/CCKB receptor gene expression by oncogenic ras in human colon cancer cell lines LoVo and Colo320HSR, showing that the gastrin/CCKB receptor may be involved in the cell-proliferative effect of oncogenic ras.

REFERENCES

1. Ishizuka J, Townsend Jr CM, Bold RJ, Martinez J, Rodriguez M, Thompson JC. Effects of gastrin on 3',5'-cyclic adenosine monophosphate, intracellular calcium, and phosphatidylinositol hydrolysis in human colon cancer cells. Cancer Res 1994;54:2129-35.
2. Gilligan CJ, Lawton GP, Tang LH, West AB, Modlin IM. Gastric carcinoid tumors: the biology and therapy of an enigmatic and controversial lesion. Am J Gastroenterol 1995;90:338-52.
3. Bordi C, D'Adda T, Azzoni C, Pilato FP, Caruana P. Hypergastrinemia and gastric enterochromaffin-like cells. Am J Surg Pathol 1995;19(suppl 1):S8-19.
4. Konturek PC, Hartwich A, Zuchowicz M, Labza H, Pierzchalski P, Karczewska E, et al. *Helicobacter pylori*, gastrin and cyclooxygenases in gastric cancer. J Physiol Pharmacol 2000;51:737-49.
5. Xu Y, Kaji H, Okimura Y, Matsui T, Abe H, Chihara K. Paracrine stimulation of cell growth by cholecystokinin/gastrin through cholecystokinin-B receptor on GH3 cells in vitro. Neuroendocrinology 1996;64:280-5.
6. Iwata N, Murayama T, Matsumori Y, Ito M, Nagata A, Taniguchi T, et al. Autocrine loop through cholecystokinin-B/gastrin receptors involved in growth of human leukemia cells. Blood 1996;88:2683-9.
7. Orbuch M, Venzon DJ, Lubensky IA, Weber HC, Gibril F, Jensen RT. Prolonged hypergastrinemia does not increase the frequency of colonic neoplasia in patients with Zollinger-Ellison syndrome. Dig Dis Sci 1996;41:604-13.
8. Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J. Gastrin and colorectal cancer: a prospective study. Gastroenterology 1998;115:275-80.
9. Wank SA, Pisegna JR, de Weert A. Brain and gastrointestinal

cholecystokinin receptor family: structure and functional expression. Proc Natl Acad Sci U S A 1992;89:8691-5.
10. Matsushima Y, Kinoshita Y, Nakata H, Inomoto-Naribayashi Y, Asahara M, Kawanami C, et al. Gastrin receptor gene expression in several human carcinomas. Jpn J Cancer Res 1994;85:819-24.
11. Smith AM, Watson SA. Gastrin and gastrin receptor activation: an early event in adenoma-carcinoma sequence. Gut 2000;47:820-24.
12. Smith AM, Watson SA. Review article: gastrin and colorectal cancer. Aliment Pharmacol Ther 2000;14:1231-47.
13. White MA, Nicolette C, Minden A, Polverino A, Van Aelst L, Karin M, et al. Multiple Ras functions can contribute to mammalian cell transformation. Cell 1995;80:533-41.
14. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-67.
15. Nakata H, Wang S, Chung DC, Westwick JK, Tillotson LG. Oncogenic ras induces gastrin gene expression in colon cancer. Gastroenterology 1998;115:1144-53.
16. Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC. Gastrin is a target of the β-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. J Clin Invest 2000; 106:533-9.
17. Seva C, Dickinson CJ, Yamada T. Growth-promoting effects of glycine-extended progastrin. Science 1994;265:410-2.
18. Singh P, Xu Z, Dai B, Rajaraman S, Rubin N, Dhruva B. Incomplete processing of progastrin expressed by human colon cancer cells: role of non-carboxyamidated gastrin in proliferation. Am J Physiol 1994;66:G459-468.
19. Hollande F, Imdahl A, Mantamadiotis T, Ciccotosto GD, Shulkes A, Baldwin GS. Glycine-extended gastrin acts as an autocrine growth factor in a nontransformed colon cell line. Gastroenterology 1997;113:1576-88.
20. Kaise M, Muraoka A, Seva C, Takeda H, Dickinson CJ, Yamada T. Glycine-extended progastrin processing intermediates induce H+,K+-ATPase α-subunit gene expression through a novel receptor. J Biol Chem 1995;270:11155-60.
21. Singh P, Owlia A, Espeijo R, Dai B. Novel gastrin receptors mediated mitogenic effects of gastrin processing intermediates of gastrin on Swiss 3T3 fibroblasts. J Biol Chem 1995;270:8429-38.
22. Romani R, Howes LG, Morris DL. Dose dependent in vivo inhibition of human colorectal cancer (LoVo) by the gastrin receptor antagonist CI-988. Clin Exp Pharmacol Physiol 1996; 23:438-40.
23. Romani R, Howes LG, Morris DL. Gastrin receptor antagonist CI-988 inhibits growth of human colon cancer in vivo and in vitro. Aust N Z J Surg 1996;66:235-7.
24. Fontana MG, Donato F, Villanacci V, Ghirardi M, Moneghini D, Di-Betta E, et al. Inhibitory effect of a gastrin receptor antagonist, CR2945, on 1, 2-dimethylhydrazine-induced colorectal cancer in mice. Eur Surg Res 1999;31:406-11.
25. Watson SA, Clarke PA, Morris TM, Caplin ME. Antiserum raised against an epitope of the cholecystokinin B/gastrin receptor inhibits hepatic invasion of a human colon tumor. Cancer Res 2000;60:5902-7.