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Nonsteroidal anti-inflammatory drugs may prevent colon cancer through suppression of hepatocyte growth factor expression

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Abstract

Nonsteroidal anti-inflammatory drugs which inhibit cyclooxygenase have been reported to suppress colon carcinogenesis. However the mechanism has not yet been elucidated. Growth factors such as hepatocyte growth factor, which are produced by fibroblasts, have been shown to be important in carcinogenesis and the progression of various human cancers. In the present study, we tested the hypothesis that nonsteroidal anti-inflammatory drugs inhibit hepatocyte growth factor expression through an endogenous prostaglandin-mediated pathway in cultured human colonic fibroblasts. Human colonic fibroblasts were obtained from a resected colon and cultured. Hepatocyte growth factor and prostaglandin E_2 were measured by enzyme-linked immunosorbent assay. Induction of cyclooxygenase-1 and cyclooxygenase-2 protein was estimated by immunoblotting. Prostaglandins increased hepatocyte growth factor production significantly in a dose- and time-dependent manner. Cholera toxin and 8-bromo cAMP also stimulated hepatocyte growth factor production. Further, prostaglandin E_1 significantly increased cellular cAMP. The prostaglandin EP_2 and EP_4 receptors were detected by reverse transcription-polymerase chain reaction. Interleukin-1β dramatically increased prostaglandin E_2 production and significantly stimulated hepatocyte growth factor synthesis. Interleukin-1β induced cyclooxygenase-2 but not cyclooxygenase-1 protein. Indomethacin significantly reduced interleukin-1β-induced prostaglandin E_2 release and hepatocyte growth factor production. These results suggest that prostaglandin is a factor for the production of hepatocyte growth factor by human colonic fibroblasts. Nonsteroidal anti-inflammatory drugs may suppress colon carcinogenesis, in part, through the suppression of hepatocyte growth factor expression by inhibiting endogenous prostaglandin production.

Keywords: NSAID (nonsteroidal anti-inflammatory drug); Colon cancer; Prostaglandin; Hepatocyte growth factor

1. Introduction

Colorectal cancer is one of the major causes of cancer death. Improved prevention, detection and treatment of this disease is an important goal in health care. At present, nonsteroidal anti-inflammatory drugs (NSAIDs) are the most promising chemopreventive agents for colorectal cancer. Several studies in animal models have shown that administration of NSAIDs results in a dramatic reduction in the size and number of intestinal tumors. In rats, azoxymethane treatment causes the development of tumors in the large intestine, although the mechanism by which azoxymethane causes colorectal neoplasia is unknown. Several NSAIDs, including indomethacin, piroxicam, aspirin, ibuprofen, and sulindac, suppress azoxymethane-induced colon carcinogenesis in laboratory animals (Reddy et al., 1987, 1990, 1992, 1993; Rao et al., 1995). Additionally, colon tumor volume was reduced in animals treated with sulindac (Rao et al., 1995). Similar preventive effects against intestinal tumors have been observed in the Min (multiple intestinal neoplasia) mouse, which exhibits a phenotype similar to that in humans with familial adenomatous polyposis and develops numerous intestinal polyps (Moser et al., 1990). One report has shown that piroxicam, a potent cyclooxygenase inhibitor, dramatically reduces tumor formation in Min mice (Jacoby et al., 1996).

The preventive effects of NSAIDs against colon carcinogenesis have also been confirmed in recent human studies. A double-blind, randomized, placebo-controlled trial of the effects of sulindac on polyp regression was performed in patients with familial adenomatous polyposis (Giardiello et al., 1993). The size and number of polyps were significantly lower in the group treated with sulindac

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compared with the control group. However, sulindac did not cause complete polyp regression in any of the patients. The fact that NSAIDs induced polyp regression in familial adenomatous polyposis patients has led to several other human studies. The largest prospective study of aspirin use and colon cancer revealed that the relative risk of colon cancer mortality was reduced in men and in women who took aspirin (Thun et al., 1991).

These experimental and epidemiologic studies suggest a role for NSAIDs in the prevention of colon carcinogenesis. However, the mechanism by which NSAIDs prevent colon cancer is still unknown. Because NSAIDs suppress cyclooxygenase and, therefore, the production of prostaglandins, cyclooxygenase might be an important molecular target in the NSAIDs-mediated prevention of colon carcinogenesis. cyclooxygenase-1 is a constitutively expressed gene and cyclooxygenase-2 is an inducible isoform of the enzyme (Herschman, 1994). Recently, cyclooxygenase-2 mRNA has been reported to be over-expressed in human colon adenomas and carcinomas (Eberhart et al., 1994). Additionally, cyclooxygenase-2, but not cyclooxygenase-1, protein is specifically expressed in human colon cancer (Kargman et al., 1995). It was demonstrated that normal intestinal epithelial cells transfected with cyclooxygenase-2 gained phenotypic changes similar to those found in malignant cells, such as resistance to apoptosis (Tsujii and Dubois, 1995). NSAIDs, including sulindac, have been found to induce apoptosis in human colon cancer HT-29 cells (Schiff et al., 1995). These studies suggest that inhibition of cyclooxygenase-2 by NSAIDs with subsequent induction of apoptosis may be involved in the prevention of colon carcinogenesis. However, the importance of this step remains to be elucidated.

One study has revealed that a cyclooxygenase-2 gene knockout mutation introduced into a $APC^{\Delta 716}$ knockout mouse dramatically reduced the rate of intestinal polyp formation (Oshima et al., 1996). Therefore, cyclooxygenase-2 may play an essential role in intestinal polyp formation. Interestingly, immunohistochemical studies have shown that cyclooxygenase-2 expression is present not in tumor cells but rather in nonepithelial cells. This has led to the hypothesis that the target of NSAIDs action is nonepithelial cells.

It has been reported that fibroblasts or paracrine factors produced by fibroblasts, are important in the regulation of cancer cell behavior (Camps et al., 1990). Hepatocyte growth factor (HGF), a growth factor produced by fibroblasts, has been shown to be involved in tumor growth and invasiveness (Iwazawa et al., 1996). HGF has been shown to modulate the cellular function and invasiveness of colon cancer cell lines (Nursat et al., 1994; Sunitha et al., 1994). Further, its receptor, c-met, is over-expressed on colon cancer cell lines and in human colon cancer tissues (Di Renzo et al., 1991; Kermorgant et al., 1996). Therefore, HGF and c-met may play important roles in colon carcinogenesis. Recent studies using primary cultured skin (Matsumoto et al., 1995) and gastric fibroblasts (Takahashi et al., 1996) suggested that prostaglandin is a potent inducer of HGF expression. Based on the above evidence, we hypothesized that NSAIDs inhibit HGF expression through an endogenous prostaglandin-mediated pathway. In the present study, effects of prostaglandin on HGF production, regulation of endogenous prostaglandin production and COX-2 expression, and the effects of indomethacin on prostaglandin and HGF production were studied in cultured human colonic fibroblasts.

2. Materials and methods

2.1. Reagents

PGE_1, PGE_2, OP2507 (a PGI_2 analog) were kindly donated by Ono Pharmaceutical (Osaka, Japan). Interleukin-1β was purchased from Upstate Biotechnology (Lake Placid, NY). 8-Bromo-cAMP and cholera toxin were obtained from Sigma Chemical (St. Louis, MO). The reagents for fibroblast culture included: Coon's modified Ham's F-12 medium (F-12 medium, Sigma), Basal Eagle's medium (BME), Minimal Essential Medium (MEM; Sigma), bovine serum albumin (fraction V; Sigma), Hanks' balanced salt solution (HBSS; Gibco Laboratories, Grand Island, NY), pronase (Kaken Pharmaceutical, Tokyo, Japan), crude type I collagenase (Sigma), and EDTA (Sigma). HGF enzyme-linked immunosorbent assay (ELISA) kit was obtained from Otsuka Pharmaceutical (Tokyo, Japan). The prostaglandin E_2 ELISA kit and cAMP ELISA kit were purchased from Cayman Chemical (Ann Arbor, MI).

2.2. Cell culture

Colonic mucosa was obtained at the time of surgery. The submucosa was separated with scissors and the mucosal layer was incubated in BME containing pronase (10 mg/ml) for 10 min at 37°C. Mucus was removed with gauze and the mucosa was minced into 2–3 mm^2 pieces, which were incubated in BME containing crude type I collagenase (0.4 mg/ml) for 15 min. After washing with Earle's balanced salt solution containing 1 mM EDTA, the tissue was once again incubated in BME containing collagenase at 37°C for 30 min. Cells from the final incubation were washed in HBSS and the cell number was adjusted at 10^5 cells/ml in F-12 medium supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum (Gibco Laboratories), 15 mM HEPES, 100 U/ml penicillin, 100 U/ml streptomycin, and 5 μg/ml fungizone. Medium (0.5 ml) containing isolated cells was inoculated onto each well of 24-well culture plates (Falcon, NJ). Cells were cultured at 37°C in a humid 5% CO_2 atmosphere. The culture was maintained for at least 1 month, after which time the mucosal epithelial cells died and the fibrob-

lasts became predominant cell type. During culture, F-12 medium supplemented with 10% FBS was changed twice a week. Subculture was performed up to four times if necessary, using trypsin and EDTA (Sigma).

2.3. Cell viability

Cell viability was estimated by the trypan blue dye exclusion test. 0.2% trypan blue solution was added to the cells, and nonstained and stained cells in a given area were counted.

2.4. Determination of HGF protein

In order to measure the production of HGF by cultured fibroblasts, the culture medium was aspirated and the monolayers of cells were rinsed twice with HBSS. F-12 medium alone or with test reagents was then layered on each cell monolayer, and the cells were incubated. The incubation medium was collected and stored at −20°C. HGF was measured by ELISA using monoclonal antibody directed against human HGF (Otsuka).

2.5. Determination of prostaglandin E_2

To measure the production of prostaglandin E_2 by cultured fibroblasts, the culture medium was aspirated and the monolayers were rinsed twice with HBSS. After rinsing, F-12 medium, either alone or with interleukin-1β, was placed on each monolayer. The monolayers were then incubated and the incubation medium was collected and stored at −20°C. The content of prostaglandin E_2 in the media was measured directly using prostaglandin E_2 ELISA kits (Cayman).

2.6. Determination of cAMP

After incubation for 1 h with F-12 medium either alone or with prostaglandin E_1, the medium was decanted off and cAMP was extracted from the cells by adding 0.5 ml of ice cold 5% (v/v) trichloroacetic acid to each culture. After 10 min at 4°C, the cells were sonicated and centrifuged at $1000 \times g$ for 15 min. The supernatant was extracted twice with 10 vols. of water-saturated diethyl ether. The cAMP concentrations were measured using a cAMP ELISA kit (Cayman).

2.7. Reverse transcription-polymerase chain reaction for EP2 and EP4 prostaglandin receptors

Total cellular RNA was isolated from cultured human colonic fibroblasts grown to confluence in 100 mm culture plates using RNAzol™ B (TEL-TEST, Friendswood, TX). Polymerase chain reaction was carried out in a reaction buffer containing 50 mM bicine, 115 mM potassium acetate, 8% (w/v) glycerol (pH 8.2), 2.5 mM MgCl2, 300 μM each of dATP, dGTP, dCTP, and dTTP, and 5 U of r*Tth* DNA polymerase (Perkin Elmer, Branchburg, NJ). One microgram of total RNA was reverse transcribed at 60°C for 30 min. Following reverse transcription, the product was denatured at 94°C for 1 min. Forty cycles of amplification of the EP_2 and EP_4 first-strand cDNA were performed with 0.45 μM of each EP_2 primer (sense: 5′-^{1198}ACACAGTCAGATGCCAGTAA^{1217}-3′; antisense: 5′-^{1721}AA-ATAAGGGTTCGCTAAGT^{1702}-3′; Regan et al., 1994), and EP_4 primers (sense: 5′-^{527}AAGGAGCAGAAGGAGACGAC^{546}-3′; antisense: 5′-^{984}GTGGCGAGAATGAGGAAGGA^{965}-3′; Bastin et al., 1994). Each amplification cycle consisted of first denaturation at 94°C for 60 s and subsequent denaturation at 94°C for 15 s, and annealing and polymerization at 60°C for 30 s. Polymerase chain reaction products (10 μl) were electrophoretically separated on 2% agarose gel in 1× TAE buffer and identified by ethidium bromide staining (0.5 μg/ml). U-937 cells, which are known to express EP_2 and EP_4 prostaglandin receptors (Blaschke et al., 1996), were used as positive control in the detection of mRNA expression of these receptors by reverse transcription-polymerase chain reaction. To monitor cDNA synthesis, reverse transcription-polymerase chain reaction for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also performed using primers (sense: 5′-^{59}CCAGCCGAGCCACATCGCTC^{68}-3′; antisense: 5′-^{408}ATGAGCCCCAGCCTTCTCCAT^{388}-3′; Soeth et al., 1996).

2.8. Western blot analysis of cyclooxygenase protein

Cells were incubated with or without interleukin-1β and then washed and lysed in 1 ml of ice-cold extraction buffer. After 30 min, the cells were scraped and centrifuged at $17{,}000 \times g$ for 10 min at 4°C. The supernatant was collected and the protein content was determined using a modified Lowry's method using a BIO-RAD (Hercules, CA) DC protein assay kit. Cell lysate was

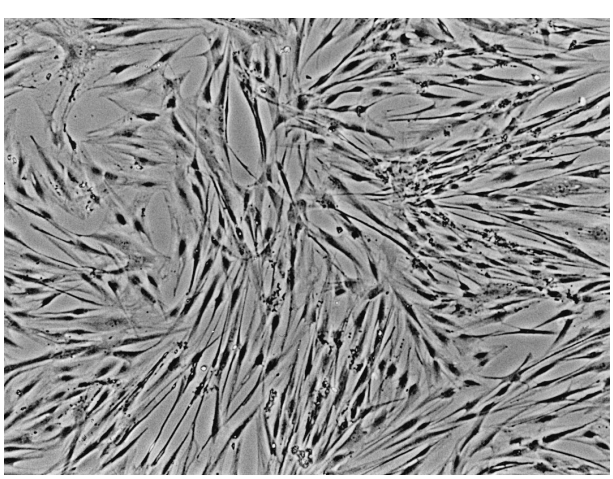

Fig. 1. Representative phase-contrast micrograph of human colonic fibroblasts in culture (×100).

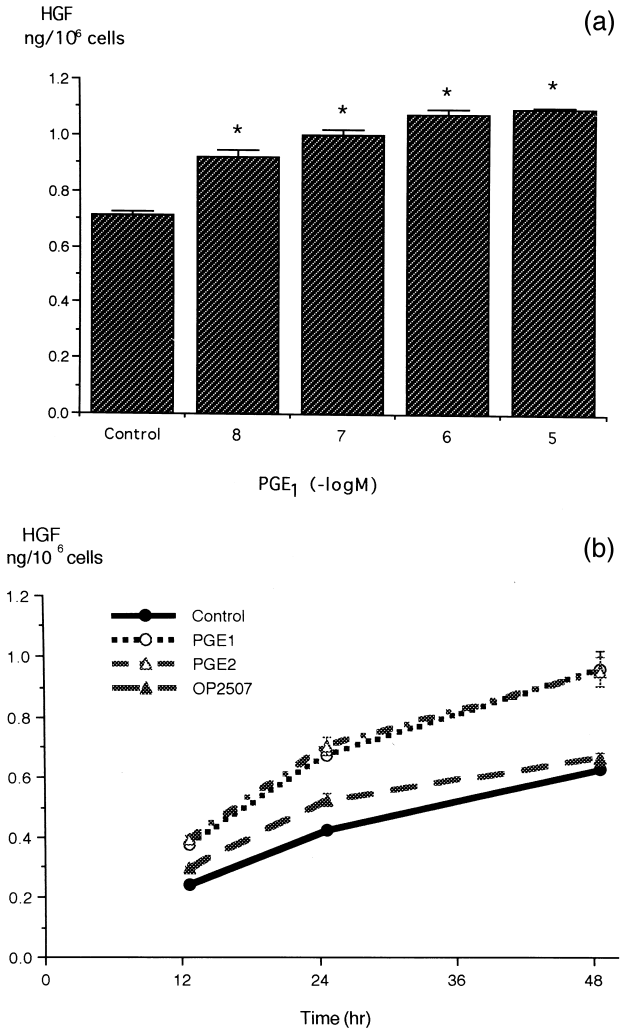

Fig. 2. Effects of 24 h treatment with prostaglandins on HGF production by human colonic fibroblasts. (a) HGF production induced by PGE_1. (b) Time course for HGF production induced by prostaglandins (10^{-6} M). HGF: hepatocyte growth factor, PG: prostaglandin. Values reported as the means ± S.E., $* P < 0.01$ vs. control.

mixed with Laemmli reagent and heated for 3 min. Equal amounts of protein were run on 10% sodium dodecyl sulfate-polyacrylamide gel. Proteins were then transferred to pure nitrocellulose membranes and the membranes were blocked overnight at 4°C with 5% BSA in Tris-buffered saline with 0.1% Tween 20 (TBS-T). The membranes were then incubated with purified polyclonal rabbit IgG antibody directed against human cyclooxygenase-2 (1:1000, Cayman) or purified monoclonal mouse IgG antibody directed against human cyclooxygenase-1 (1:1000, Cayman) for 1 h at room temperature. The immunoblots were washed in TBS-T and incubated for 1 h at room temperature with donkey anti-rabbit IgG antibody (cyclooxygenase-2) or sheep anti-mouse IgG (cyclooxygenase-1) coupled to horseradish peroxidase (Amersham, Arlington Heights, IL) at 1:2000 dilution in TBS-T. An Enhanced Chemiluminescence (ECL) kit (Amersham) was used for the detection of protein.

2.9. Statistical analysis

Data are presented as the means ± S.E. Comparisons between two groups were performed using Student's *t*-test for grouped or paired data where appropriate. Comparisons between several groups were performed by analysis of variance, followed by Dunnett's test, where appropriate. In all analyses, statistical significance was defined as $P < 0.05$.

3. Results

3.1. Human colonic mucosal fibroblast culture

Cell culture of colonic cells was relatively more difficult than that of gastric cells mainly due to bacterial contamination. This was mostly prevented by the pretreat-

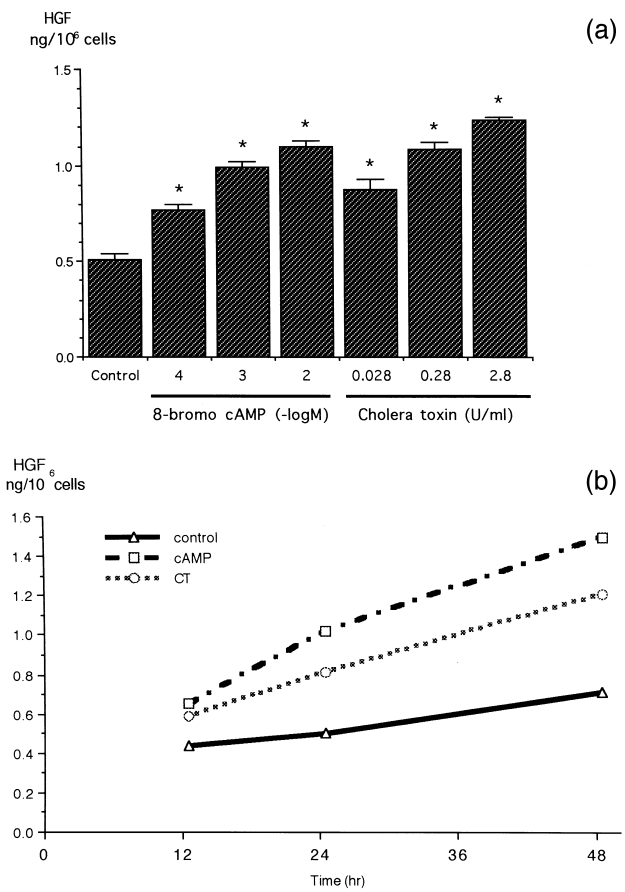

Fig. 3. Effects of 24 h incubation with 8-bromo cAMP and cholera toxin on HGF production by human colonic fibroblasts. (a) Dose-dependent HGF production. (b) Time course for HGF production induced by either 10^{-3} M of cAMP or 2.8 U/ml of cholera toxin. HGF: hepatocyte growth factor, CT: cholera toxin. Values reported as the means ± S.E., $* P < 0.01$ vs. control.

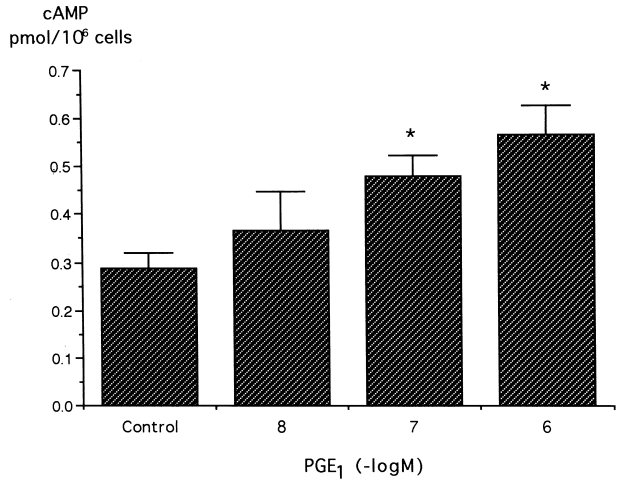

Fig. 4. Cellular cAMP formation by human colonic fibroblasts incubated for 1 h with PGE1. PG: prostaglandin. Values reported as the means ± S.E., * $P < 0.01$ vs. control, * * $P < 0.05$ vs. control.

ment with pronase. Cultured cells formed monolayers after about one month. After one month, the cells were subcultured after treatment with trypsin EDTA. Fig. 1 shows a representative phase-contrast micrograph of confluent colonic fibroblasts.

3.2. Cell viability

The viability of cultured cells as assessed by trypan blue dye exclusion test after 48 h was over 95%. None of the agents used in the present study affected the viability or number of these cells (data not shown).

3.3. Effect of prostaglandins on HGF production

Prostaglandin E_1 significantly increased HGF production in a dose-dependent fashion (Fig. 2a). Prostaglandin E_2 and OP2507 showed similar effects (data not shown). Fig. 2b shows the time course for HGF production. The order of potency for prostaglandin-induced HGF production was prostaglandin E_1 = prostaglandin E_2 < OP2507.

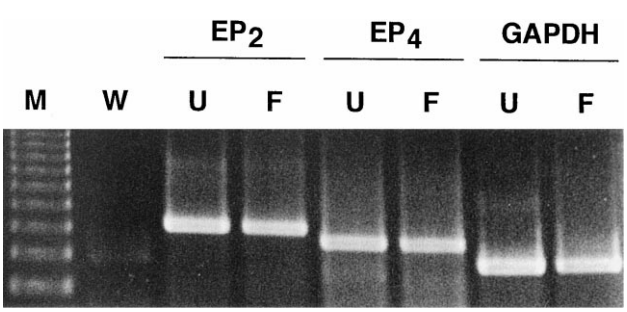

Fig. 5. Expression of the EP_2 and EP_4 prostaglandin receptors by human colonic fibroblasts as assessed by reverse transcription-polymerase chain reaction. M: marker, W: water (negative control), U: U-937 cells (positive control), F: human colon fibroblasts, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

3.4. Effect of exogenous cAMP and cholera toxin on HGF production

EP_2 and EP_4 prostaglandin receptors stimulate adenylate cyclase-mediated increases in cAMP while EP_3 prostaglandin receptors induce opposite effects (Coleman et al., 1990). To elucidate the mechanism by which prostaglandins induce HGF production, the effects of exogenous and endogenous cAMP on HGF production were investigated. Membrane-permeable 8-bromo-cAMP and cholera toxin, a stimulator of adenylate cyclase, significantly increased HGF production by colonic fibroblasts in a dose- and time-dependent manner (Fig. 3). These findings suggest that prostaglandins induce HGF production by activating adenylate cyclase.

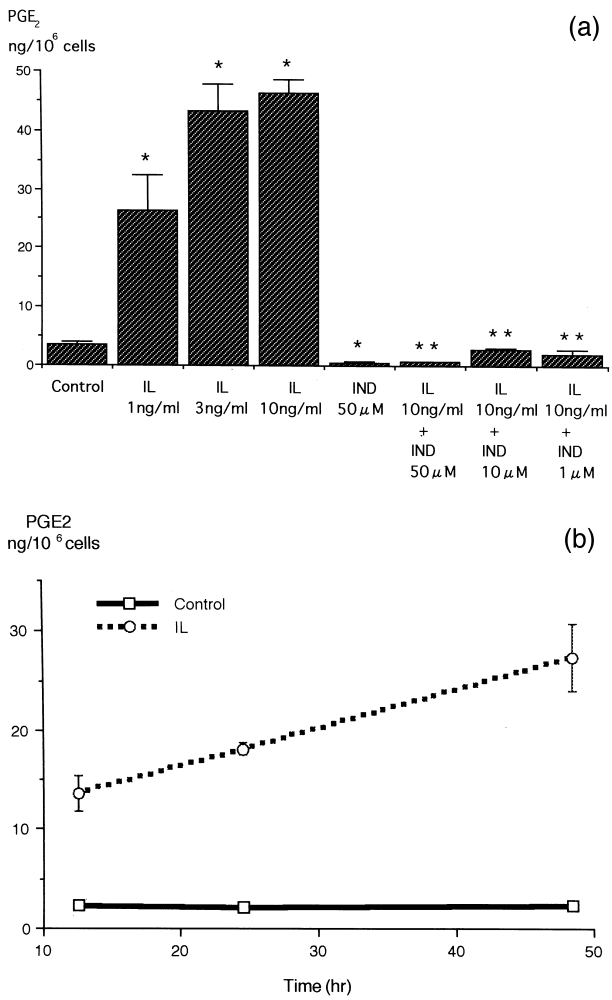

Fig. 6. Effects of 24 h treatment with interleukin-1β and indomethacin on prostaglandin E_2 production by human colonic fibroblasts. (a) Dose-dependent prostaglandin E_2 production. (b) Time course for prostaglandin production induced by interleukin-1β. IL: interleukin-1β, PG: prostaglandin, IND: indomethacin. Values reported as the means ± S.E., * $P <$ 0.01 vs. control; * * $P < 0.01$ vs. IL 10 ng/ml.

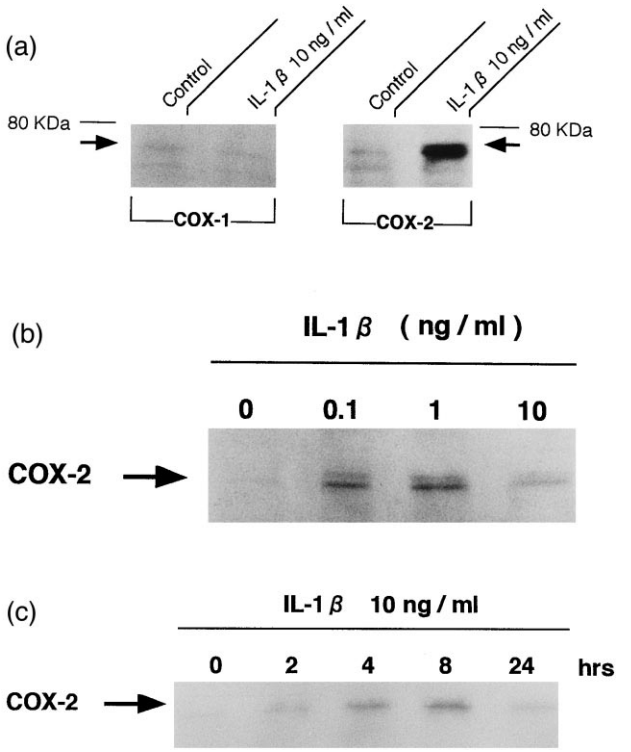

Fig. 7. Effects of interleukin-1β on COX protein expression as assessed by western blotting. (a) Effect of interleukin-1β on COX-1 and COX-2. Cells were incubated for 4 h with interleukin-1β (10 ng/ml). (b) interleukin-1β-induced increases in COX-2 protein. Cells were incubated for 8 h with interleukin-1β. (c) Time course for interleukin-1β-induced increases in COX-2 protein. Cells were incubated with interleukin-1β (10 ng/ml) for the indicated times. 30 μg protein/lane for (a), or 10 μg protein/lane for (b) and (c) were run on 10% polyacrylamide gels. COX: cyclooxygenase, IL: interleukin-1β.

3.5. Effect of prostaglandin E_1 on endogenous cAMP formation

In order to confirm that the action of prostaglandin is mediated by cAMP, we examined the effects of prostaglandin E1 on the formation of cAMP by colonic fibroblasts. As shown in Fig. 4, prostaglandin E_1 significantly increased cAMP in a dose-dependent manner, suggesting that the increase in HGF production by prostaglandins is mediated by a cAMP-dependent pathway.

3.6. Expression of EP_2 and EP_4 prostaglandin receptors by colonic fibroblasts

Because prostaglandins appeared to stimulate HGF production through an increase in cAMP formation, the expression of EP_2 and EP_4 mRNA was studied. Reverse transcription-polymerase chain reaction clearly revealed the expression of EP_2 and EP_4 mRNA in colonic fibroblasts (Fig. 5), although the expression of these receptors after the treatment with interleukin-1β was not studied. These results suggested that prostaglandins induce HGF expression by increasing cAMP formation through the specific prostaglandin receptors EP_2 and/or EP_4 in human colonic fibroblasts.

3.7. Effect of interleukin-1β on prostaglandin E_2 production

We investigated the effects of growth factors, such as fibroblast growth factor, platelet-derived growth factor, and transforming growth factor-α, and of cytokines, such as tumor necrosis factor-α and IL1-α on prostaglandin E_2 production. Of these agents only interleukin-1β increased prostaglandin E_2 under serum-free condition (data not shown). As shown in Fig. 6, interleukin-1β significantly increased prostaglandin E_2 production in a dose- and time-dependent manner. Indomethacin at a concentration greater than 10^{-6} M completely abolished the interleukin-1β-induced increase in prostaglandin E_2 production.

3.8. Effect of interleukin-1β on cyclooxygenase protein expression

In order to elucidate the mechanism by which interleukin-1β increases prostaglandin E_2 production by colonic fibroblasts, the expression of cyclooxygenase, a key enzyme in prostaglandin synthesis, was studied. Interleukin-1β at a concentration of 0.1–10 ng/ml induced cyclooxygenase-2 but not cyclooxygenase-1 expression after 4 h (Fig. 7a and b). Interleukin-1β increased the expression of cyclooxygenase-2 for up to 8 h and cyclooxygenase-2 expression was then decreased after 24 h (Fig. 7c). These results indicate that interleukin-1β stimulates prostaglandin E_2 production through the induction of cyclooxygenase-2.

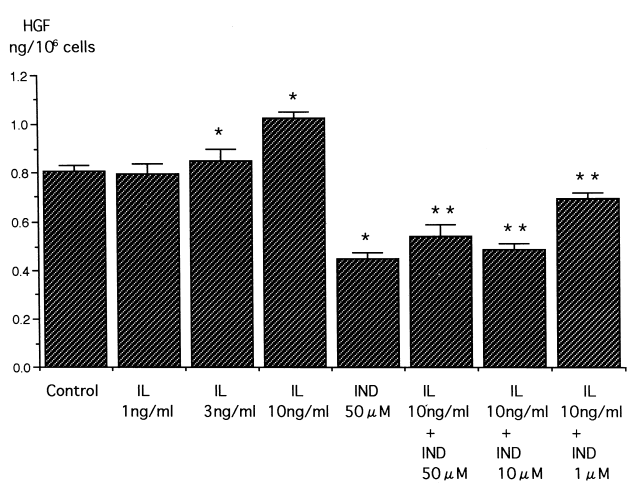

Fig. 8. Effects of interleukin-1β and indomethacin of HGF production by human colonic fibroblasts. Cells were incubated for 24 h. HGF: hepatocyte growth factor, IL: interleukin-1β, PG: prostaglandin, IND: indomethacin. Values reported as the means ± S.E., $*\ P < 0.01$ vs. control, $**\ P < 0.01$ vs. IL 10 ng/ml.

3.9. Effect of interleukin-1β and indomethacin on HGF production

Interleukin-1β significantly increased the production of HGF by colonic fibroblasts in a dose-dependent fashion. In contrast, indomethacin significantly decreased basal and interleukin-1β-induced HGF production (Fig. 8).

4. Discussion

NSAIDs including indomethacin have been known to suppress colon carcinogenesis, however, the cellular and biochemical mechanisms responsible for this dramatic effect have not been established. Because NSAIDs are cyclooxygenase inhibitors, their effects might be mediated by suppression of this enzyme and inhibition of prostaglandin synthesis. Accumulating evidence suggests that human colon cancers and adenomas have increased cyclooxygenase-2 activity (an inducible isoform of cyclooxygenase; Eberhart et al., 1994; Kargman et al., 1995). Therefore, previous studies have focused on the role of cyclooxygenase-2, and tumor cell apoptosis mediated by the inhibition of cyclooxygenase-2 by NSAIDs (Tsujii and Dubois, 1995; Schiff et al., 1995). Recently, cyclooxygenase-2 was shown to be expressed in adenomas of $APC^{\Delta 716}$ knockout mice at a very early stage of intestinal polyp formation. Interestingly, the cyclooxygenase-2 was only expressed in nonepithelial cells but not in tumor cells (Oshima et al., 1996). Therefore, an interaction between nonepithelial cells and tumor cells may be an essential factor in intestinal polyp formation. Studies concerning the biology of nonepithelial cells are required to clarify the role of cyclooxygenase-2 in intestinal polyp formation. These facts lead us to investigate the biology of human colonic fibroblasts, because fibroblasts produce growth factors and cytokines which might be related to tumor growth and extension.

Human colonic fibroblasts produce HGF, which has been shown to be involved in growth and invasion of human malignancy including colon cancer (Di Renzo et al., 1991; Nursat et al., 1994; Sunitha et al., 1994; Iwazawa et al., 1996; Kermorgant et al., 1996). Prostaglandin E_1, prostaglandin E_2, and OP2507, a prostaglandin I_2 analog, increased HGF production by colonic fibroblasts as shown in Fig. 2. These results are in keeping with previous studies of the regulation of HGF in human skin and gastric fibroblasts (Matsumoto et al., 1995; Takahashi et al., 1996). However, the potency of the various of prostaglandins for HGF production by colonic fibroblasts in our study is somewhat different from the potency of the prostaglandin in fibroblasts in skin or stomach. This would suggest that there is fibroblast organ specificity. The actions of prostaglandin seem to be mediated by the specific prostaglandin receptors, EP_2 and/or EP_4. This is based on our findings that exogenous cAMP and cholera toxin caused effects similar to those of prostaglandins, combined with the fact that prostaglandin E_1 increased cAMP formation and that the mRNA for these receptors was detected by reverse transcription-polymerase chain reaction. Our findings suggest that prostaglandin receptor antagonists may prove to be an important anti-cancer therapy, although further studies such as the expression of these receptors after the stimulation with interleukin-1β or the effect of receptor antagonists on HGF production are required. Our results also suggest that a prostaglandin-dependent pathway may be crucial in the NSAID-induced chemoprevention of colon cancer.

We investigated the production of prostaglandin E_2, which is a major prostaglandin generated in the colon (Rigas et al., 1993), and the induction of cyclooxygenase. Of several potential stimulants, only interleukin-1β dramatically increased prostaglandin E_2 production (Fig. 6). Interleukin-1β also induced cyclooxygenase-2, but not cyclooxygenase-1, protein synthesis (Fig. 7). Therefore, induction of cyclooxygenase-2 expression may be involved in the interleukin-1β-induced increase in prostaglandin E_2 in human colonic fibroblasts. The expression of cyclooxygenase-2 was decreased after 24 h, although there was a gradual increase of prostaglandin E_2 in the culture media up to 48 h. Therefore, the accumulation of prostaglandin E_2 in the media persisted during down regulation of cyclooxygenase-2. The precise mechanisms and explanation of this phenomenon require further studies. While interleukin-1β has been shown to have these effects on prostaglandin synthesis in cells from other organs (Ben-Av et al., 1995; Akarasereenont and Thiemermann, 1996), the regulation of prostaglandin and cyclooxygenase has not been studied extensively in the colon. Because cyclooxygenase-2 plays a crucial role in colon carcinogenesis, the mechanism responsible for the induction of this protein is an important target for cancer research.

In our study, interleukin-1β modestly increased HGF production (Fig. 8). More dramatic increases in HGF have been reported in skin fibroblasts (Matsumoto et al., 1992). This difference may be due to the origin of the fibroblasts. Indomethacin completely abolished the interleukin-1β-induced increases in prostaglandin E_2 and HGF production. We previously reported similar effects of indomethacin on HGF production by human gastric fibroblasts (Bamba et al., 1998). These results confirm the importance of the prostaglandin-HGF pathway in the prevention of colon cancer by NSAIDs.

In conclusion, this report demonstrated that prostaglandin is a mediator of HGF production by human colonic fibroblasts. Further, NSAIDs may suppress colon carcinogenesis, in part, through the suppression of HGF expression by inhibiting endogenous prostaglandin production.

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