Prostaglandin E₂ Enhances Intestinal Adenoma Growth via Activation of the Ras-Mitogen–Activated Protein Kinase Cascade

Dingzhi Wang,¹ F. Gregory Buchanan,¹ Haibin Wang,² Sudhansu K. Dey,³,4 and Raymond N. DuBois⁺

Departments of Medicine, Pediatrics, Cancer Biology, and Cell and Developmental Biology, Vanderbilt University Medical Center and Vanderbilt-Ingram Cancer Center, Nashville, Tennessee

Abstract

A large body of clinical, genetic, and biochemical evidence indicates that cyclooxygenase-2 (COX-2), a key enzyme for prostanoid biosynthesis, contributes to the promotion of colorectal cancer. COX-2–derived prostaglandin E₂ (PGE₂) is the most abundant prostaglandin found in several gastrointestinal malignancies. Although PGE₂ enhances intestinal adenoma growth in Apcmin mice, the mechanism(s) by which it accelerates tumor growth is not completely understood. Here we investigated how PGE₂ promotes intestinal tumor growth and the signaling pathways responsible for its effects. We observed that PGE₂ treatment leads to increased epithelial cell proliferation and induces COX-2 expression in intestinal adenomas. Furthermore, we show that PGE₂ regulation of COX-2 expression is mediated by activation of a Ras-mitogen–activated protein kinase signaling cascade. One intriguing finding is that COX-2–derived PGE₂ mimics the effects of constitutively active Ras through a self amplifying loop that allows for a distinct growth advantage. (Cancer Res 2005; 65(5): 1822-9)

Introduction

Colorectal cancer develops according to a complex and multistep process which involves genetic alterations and progressive changes in signaling pathways that regulate intestinal epithelial cell proliferation, differentiation, and apoptosis. A large body of data supports a role of the cyclooxygenase-2 (COX-2) enzyme in colorectal cancer progression (1, 2) and indicates that inhibition of COX-2 reduces tumor growth (3). Moreover, it is well established that cyclooxygenase enzymes play a key role in intestinal adenoma formation in Apcmin mice, a model frequently employed for studying colorectal cancer (4), and in ApcΔ716 mutant mice (5). Tumor number decreases when Apc mutant mice are treated with COX-2-selective inhibitors (5). In addition, in vitro studies confirm that expression of COX-2 is correlated with intestinal epithelial cell proliferation and invasiveness, a reduction in the apoptotic rate, and induction of proangiogenic factors such as vascular endothelial growth factor (6–9).

COX-2 is an inducible gene which is regulated by a number of factors, including serum, growth factors, proinflammatory cytokines, hormones, oncogenes, or tumor promoters (10). Various extracellular stimuli regulate COX-2 expression through a mitogen-activated protein kinase (MAPK)–dependent pathway. For example, transforming growth factor α, INFγ, and platelet-derived growth factor induce COX-2 expression via activation of the extracellular signal–regulated kinase (ERK) signal transduction pathway in normal human epidermal keratinocytes, squamous carcinoma cells, and NIH 3T3 cells, respectively (11, 12).

COX-2 is the key enzyme for the conversion of arachidonic acid to prostaglandins, such as PGE₂, PGD₂, PGF₂α, and PGI₂ (10, 13–15). PGE₂ is the most abundant prostaglandin found in human colorectal cancers, premalignant lesions, and cells derived from a number of solid malignancies (16–19). PGE₂ exerts its actions either in autocrine or in paracrine fashion via binding to G-protein coupled receptors (EP1–4), which belong to the family of rhodopsin-type receptors. Genetic studies using mice lacking the PGE₂ cell surface receptors EP1, EP2, or EP4 point to an important role for all three receptors in intestinal polyp formation (20–22). Moreover, EP1 or EP4 receptor antagonists decrease the incidence of intestinal adenomas in both the Apcmin and the carcinogen-treated mouse models (20, 21). These results provide strong evidence that PGE₂ plays a pivotal role in regulating intestinal adenoma formation. We recently reported that PGE₂ treatment increases adenoma burden in Apcmin mice (23). In addition, in vitro studies have shown that PGE₂ enhances clonogenicity and increases invasiveness of LS-174T carcinoma cells by activating the EP4-Pi3k-Akt signaling cascade (24) and that PGE₂ promotes integrin αvβ3–dependent endothelial cell adhesion and spreading (25). However, all of the mechanisms responsible for the effects of PGE₂ on intestinal adenoma growth are not known.

Ras mutations are found in a wide variety of human malignancies and in about 50% of colorectal cancers (26). In rodents, AOM-induced colonic carcinogenesis involves activation of the K-Ras gene (27). For example, K-Ras mutations were identified in 14 of 84 AOM-induced colonic tumors. Most importantly, a subset of tumors (18 of 70) had significantly higher activation of wild-type K-Ras compared with controls, suggesting that the activation of wild-type Ras is also involved in AOM-induced colonic carcinogenesis. Moreover, forced expression of constitutively active Ras (mutant Ras) up-regulates COX-2 expression and enhances cell proliferation in a variety of cell culture models (28–31). Therefore, we examined whether PGE₂ activates endogenous wild-type Ras that is known to regulate cell proliferation.
It is well known that activation of Ras triggers the downstream signaling pathways such as the Raf/MAPK kinase (MEK)/ERKs and PI3K/Akt pathways. In the Raf/MEK/ERKs pathway, activated Ras recruits Raf to the plasma membrane, which leads to phosphorylation of MEK, a dual specificity kinase that phosphorylates the Thr-X-Tyr motif in the activation loop of ERK. Upon activation, ERK translocates to the nucleus and regulates the activity of many transcription factors including Elk-1 (32, 33). Elk-1 is a member of the ternary complex factor family of Ets domain proteins that bind to serum response elements, a cis-element responsible for activation of immediate-early gene expression following mitogenic stimulation (34). It has been reported that serum, growth factors, and phorbol 12-myristate 13-acetate stimulate the phosphorylation of Elk-1 via the Raf-MEK-ERK pathway (35). Moreover, other seven transmembrane spanning G-protein-coupled receptor agonists have been shown to activate a Ras-MAPK signaling pathway (36). Because cytokines induce COX-2 expression by activating the ERK signal transduction pathway, we postulated that PGE2 could up-regulate COX-2 through activation of Ras-MAPK signaling.

To investigate the mechanism responsible for the effect of PGE2 to promote intestinal adenoma growth, we examined whether this lipid mediator stimulates intestinal epithelial cell proliferation and determined the downstream targets of PGE2 using both in vivo and in vitro models. Here we show that PGE2 enhances intestinal cell proliferation and induces COX-2 expression in Apcmin mouse adenomas. We further show that activation of Ras-MAPK pathway is required for PGE2 to induce COX-2 expression and stimulate HCA-7 cell proliferation. Consistent with these findings, we also observed that PGE2 enhances ERK and Elk-1 activity in intestinal adenomas and phospho-Elk-1 levels are dramatically elevated in human colorectal cancers compared with matched normal mucosa. To our knowledge, this is first report that COX-2 induction by PGE2 is mediated by activation of the Ras-MAPK pathway.

Materials and Methods

Animals. C57BL/6J-Apcmin male mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age. After 1 week, Apcmin mice (n = 6) were randomly assigned to either a control or treatment group (vehicle and 150 μg PGE2 per mouse). Vehicle or PGE2 in 100 μl sterile PBS was given twice daily via gavage feeding. After treatment for 7 weeks, mice were injected with 0.25 ml of BrdUrd labeling reagent (Zymed Laboratories, Inc., South San Francisco, CA) and sacrificed after 3 hours. The intestines were collected, opened longitudinally, fixed, and embedded in paraffin as reported previously (23). Sections were then used for cell proliferation assays and immunohistochemical staining.

In vivo Cell Proliferation Assays and Immunohistochemical Staining. Proliferating cells in tissue sections were detected using an anti-BrdUrd antibody according to the instructions for ZYMED BrdUrd kit (Zymed Laboratories). In addition, tissue sections also were stained with mouse monoclonal antibodies against phospho-Elk-1 (Ser383) or phospho-ERK1/2 (Tyr204) at a dilution of 1:250 (Santa Cruz Biotechnology, Santa Cruz, CA). The immunohistochemical staining was completed by using a Zymed-Histostain-SP Kit (Zymed Laboratories).

Cell Culture. HCA-7 cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum and penicillin-streptomycin. To arrest cell growth, cells were cultured in the absence of serum for 24 hours. The MEK1 (P890859), the Ras (Fase inhibitor III), and PI3K (LY294002) inhibitors (Calbiochem-Novabiochem Co., San Diego, CA) were prepared as a stock in DMSO (50 μmol/L).

Cell Proliferation ELISA and Cell Growth. Cell proliferation was measured using a Cell Proliferation ELISA kit (Roche Molecular Biochemicals, Indianapolis, IN) following the manufacturer's instructions. Briefly, HCA-7 cells (1 × 104 cells per well) grown on 96-well culture plates were pretreated with either DMSO or inhibitors for 1 hour and then treated with the indicated concentration of PGE2 for 24 hours after serum starvation. The cells were then labeled with BrdUrd for an additional 6 hours. Incorporation of BrdUrd was measured colorimetrically with an ELISA reader. SpectraMax 340PC (Molecular Devices, Sunnyvale, CA). Cell vitality was measured using a trypan blue exclusion assay.

Whole Cell Extracts and Western Blot Analysis. Whole cell extracts were prepared from cells treated with either vehicle or PGE2 for the indicated times and after serum starvation for 24 hours. Western blots were done following protocols provided by Santa Cruz Biotechnology. The cells were lysed in 0.6 ml of radioimmunoprecipitation assay buffer with protease inhibitor cocktail tablets (Boehringer Mannheim Co., Indianapolis, IN) and 0.2 μmol/L sodium orthovanadate. Fifty micrograms of soluble protein were fractionated in a 10% SDS-PAGE reducing gel, and then blotted onto a 0.2-μm nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane was blocked with 5% dry milk in TBS-T buffer for 1 hour and then incubated for 12 to 16 hours at 4°C in a 1:1,000 dilution of a pan-Ras antibody (AB-3; Oncogene Research Products, Cambridge, MA), an anti-phospho-ERK1,2, the anti-phospho-Elk-1, anti-Elk-1, anti-COX-2, or anti-ERK1/2 (Santa Cruz Biotechnology) in TBS-T buffer containing 5% dry milk. After three washings with TBS-T buffer, the membrane was incubated in a 1:5,000 dilution of the appropriate antirabbit or antimouse immunoglobulin conjugated with horseradish peroxidase (Boehringer Mannheim) in TBS-T buffer with 5% dry milk for 1 hour at room temperature. After three washings with TBS-T buffer, the protein bands were detected with the enhanced chemiluminescence Western blotting detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. The blots were stripped and then reprobed with anti-ERK2 or anti-Elk-1.

Transient Transfection Assays. Cells (1.5 × 105 in 12-well plates) were transiently cotransfected with 0.2 μg of COX-2 (−327 to +59) luciferase reporter gene and 5 ng of pRL-SV40 by the LipofectAMINE Plus reagent following manufacturer's protocol (Life Technologies, Inc., Rockville, MA). For cotransfection assays, the cells were transiently cotransfected with 0.3 μg COX-2 (−327 to +59) luciferase reporter gene and 5 ng of pRL-SV40 and 0.4 μg of empty vector or dominant-negative PI3K plasmids. Three hours later, the cells were placed in fresh serum-free media and incubated for another 4 hours. The cells were then treated with PGE2 after pretreatment with vehicle or inhibitors for 1 hour. After 16 hours, cells were harvested in 1× luciferase lysis buffer. Relative light units from firefly luciferase activity was determined using a luminometer, Monolight 3010, (BD Biosciences/PharMingen, San Diego, CA) and normalized to the relative light units from Renilla luciferase using the Dual Luciferase kit (Promega, Madison, WI).

Ras Activation Assays. Ras activity was measured using a Ras Activation Assay Kit (Upstate Biotechnology, Inc., Lake Placid, NY) following the manufacturer's instructions. Briefly, quiescent cells were stimulated with PGE2 at indicated concentrations and for indicated times. Cells were washed twice with ice-cold HBS and lysed in 1× Mg2+ lysis/washing buffer containing protease inhibitor cocktail tablets (Roche Molecular Biochemicals) for 15 minutes at 4°C. Cell lysates were centrifuged at 1,000 × g for 20 minutes. The supernatants were pretreated with glutathione-Sepharose-4B beads (Amersham Pharmacia Biotech) and the protein concentrations of the supernatants were then determined (Bio-Rad). Equal amounts of samples (400 μg) were immediately affinity-precipitated using 20 μg of recombinant glutathione S-transferase-Raf-1 ras binding domain (1149) fusion proteins conjugated glutathione-Sepharose beads (Amersham Pharmacia Biotech) for 1 hour at 4°C. The precipitates were washed thrice with 1× Mg2+ lysis/washing buffer and eluted by boiling in 1× SDS-PAGE sample buffer. The proteins were separated on a 12% SDS-polycrylamide gel and then immunoblotted with pan-Ras antibody (AB-3; Oncogene Research Products). To normalize the amount of GTP-bound Ras to total amount of Ras, equal volumes of cell lysate were also subjected to Western blot analysis using the pan-Ras antibody.
Results

PGE2 Induces Epithelial Cell Proliferation and COX-2 Expression in Intestinal Adenomas. Because epithelial cell proliferation may be one of the key mechanisms by which PGE2 promotes intestinal adenoma growth, we investigated whether PGE2 would affect the adenoma epithelial cell proliferation in the Apcmin mice. We treated 6-week-old Apcmin mice with vehicle (PBS) or PGE2 for 7 weeks. Before sacrifice, mice were given an injection of BrdUrd to label the proliferating cell fraction. Significant BrdUrd uptake was seen in epithelial cells found in adenomas from mice treated with PGE2 and weak staining was found in the adenomas from mice treated with vehicle alone. B, levels of COX-2 expression in the adenomas from mice treated with PGE2. Top, immunoreactive staining (brown) for incorporated BrdUrd in epithelial cells of adenomas taken from mice treated with PGE2 and weak staining was found in the adenomas from mice treated with vehicle alone. Bar, 100 μm. Bottom, three different Western blot experiments with similar results.

Figure 1. PGE2 induces intestinal epithelial cell proliferation and COX-2 expression in adenomas. Sections of small intestine were immunostained with anti-BrdUrd antibody (A) or COX-2 antibody (B). Representative section shows strong immunoreactive staining (brown) for incorporated BrdUrd in epithelial cells of adenomas taken from mice treated with PGE2 and weak staining was found in the adenomas from mice treated with vehicle alone. B, levels of COX-2 expression in the adenomas from mice treated with PGE2. Top, immunoreactive staining (brown) for COX-2. Bar, 100 μm. Bottom, three different Western blot experiments with similar results.

PGE2-Up-Regulated COX-2 Expression Is Dependent on Ras-MAPK Activation. To further elucidate the signaling pathways...
responsible for PGE2-stimulated cell proliferation and COX-2 expression, we evaluated the effects of PGE2 in HCA-7 cells that are known to exhibit COX-2-dependent proliferation (37, 38). HCA-7 cells have been carefully evaluated as an in vitro model to investigate COX-2-dependent cell growth (37, 38). These cells express PGE2 receptors (EP1, EP3, and EP4) and contain wild type Ras. As a first step in identifying the signaling pathways involved, we examined the effect of PGE2 on HCA-7 cell proliferation and COX-2 expression. A cell proliferation assay was used to directly measure the PGE2-induced cell proliferation based on the measurement of BrdUrd incorporation during DNA synthesis. As shown in Fig. 2A, cell proliferation was stimulated following 24 hours of PGE2 treatment in a dose-dependent manner. In addition, compared with untreated cells, PGE2 treatment for 5 days increased cell number by 2-, 3.2-, 3.6-, 4.2-, and 5.2-fold at 0.001, 0.01, 0.1, 1, and 10 μmol/L, respectively (Fig. 2B). These results show that PGE2 can stimulate HCA-7 cell proliferation in a dose-dependent manner.

Next, we determined the ability of PGE2 to affect COX-2 promoter activity in HCA-7 cells. As shown in Fig. 3A, a dose-dependent increase in luciferase activity (representing COX-2 promoter activity) was observed with PGE2, but not with PGD2, cPGI2, or PGF2α. Since our previous results showed that overexpression of constitutively active Ras up-regulates COX-2 expression (29), we determined whether the Ras-MAPK cascade mediates PGE2 induction of COX-2 expression in colorectal cancer cells. Our results show that either a highly selective Ras inhibitor (Ftsase inhibitor III) or a MEK inhibitor (PD98059) blocks the PGE2-enhanced COX-2 promoter activity in a dose dependent manner, but not PI3k inhibitor (Ly294002; Fig. 3B). Overexpression of a dominant-negative Ras construct also inhibited PGE2-up-regulated COX-2 promoter activity, whereas expression of a dominant negative Ras construct in HCA-7 cells significantly inhibited the PGE2-induced COX-2 promoter activity, whereas expression of a dominant-negative Ras construct in HCA-7 cells significantly inhibited the PGE2-induced COX-2 promoter activity.
negative PI3K failed to inhibit PGE2-mediated COX-2 promoter activity (Fig. 3C). Similarly, both inhibitors of Ras and MEK inhibit PGE2-enhanced COX-2 protein expression, which is not affected by a PI3K inhibitor (Fig. 3D). Furthermore, overexpression of a dominant negative Ras also blocked both basal and PGE2-induced COX-2 expression (COX-2 levels were barely detected; Fig. 3D, bottom). These results show that COX-2 is up-regulated by its own downstream product, PGE2, and that this autoinduction is dependent on activation of the Ras-MAPK pathway.

**PGE2 Activates Ras-MAPK.** To further show that PGE2 can induce endogenous Ras (wild type) activity, HCA-7 cells were treated with PGE2 for various times following 24 hours of serum starvation. Ras activation assays were done by selective affinity precipitation of GTP-bound Ras with immobilized glutathione S-transferase-c-Raf-1 ras binding domain and Ras-GTP was detected by Western blotting with a pan-Ras antibody. We found that PGE2 increases activation of Ras in a biphasic manner (Fig. 4A). The early phase of Ras activation is dose dependent (Fig. 4B, lanes 1, 2, 4, and 6), whereas the late phase of Ras activation is independent of PGE2 (lanes 1, 3, 5, and 7). Ras activation was evident as early as 1 minute, reached a maximum level at 5 minutes followed by return to the basal level by 20 minutes after 0.1 μmol/L PGE2 treatment. However, Ras activation again increased at 180 minutes and persisted for a prolonged time (data not shown). To confirm that equivalent amounts of protein were loaded on the gel, the level of total Ras protein from all samples was examined by Western blotting. All the samples exhibited similar levels of total Ras protein (Fig. 4A-B, bottom). These results show that PGE2 stimulates Ras activation in a time-dependent manner.

Because the MAPK cascade is one of the Ras downstream signaling pathways, we next examined whether PGE2 can activate MAPK signaling. HCA-7 cells were treated with PGE2 for 5 or 180 minutes. Western blotting was then done with an antibody which recognizes the phosphorylated (activated) form of ERK1/2 and its downstream target Elk-1. As shown in Fig. 4C, PGE2 treatment enhanced phosphorylation of both ERK1/2 and Elk-1 without affecting the levels of total ERK1/2 and Elk-1 proteins. However, there is evidence that the activation of ERK by other G-protein coupled receptor agonists involves both Ras-Raf-MEK dependent and independent effects. Thus, we examined whether Ras and MEK are required for PGE2-enhanced ERK activation. HCA-7 cells were pretreated with the selective Ras and MEK inhibitors for 1 hour and then treated with PGE2 for 5 minutes. Both of the inhibitors significantly blocked the PGE2-stimulated ERK activation as well as its downstream target Elk-1 (Fig. 4D) without showing apparent signs of cytotoxicity at the concentrations and incubation times studied. Furthermore, the late phase of ERK and Elk-1 activation by PGE2 (180 minutes) was also sensitive to both inhibitors (data not shown). These results show that a Ras-Raf-MEK cascade mediates the PGE2-induced ERK and Elk-1 activation.

In determining the biological significance of a signaling pathway, results obtained in vivo are helpful to verify the data obtained from cultured cells. Thus, we examined whether PGE2 can activate the MAPK cascade in Apcmin mouse adenomas in vivo. As shown in Fig. 5A, PGE2 treatment resulted in dramatic increases in both phosphorylated ERK1/2 and Elk-1 in intestinal adenomas as determined by immunostaining. Moreover, because ERK and c-jun-NH2-kinase activities increase modestly in a subset of human colorectal carcinomas (39), we examined whether the Elk-1 activation is also increased in human colorectal cancers. Western blotting was done to examine Elk-1 activation in human colon cancers and matched normal tissues. Among the 15 pairs of human colon cancer and the matched normal mucosal samples, eight pairs showed high levels of Elk-1 activation (phosphorylation) in the cancer specimens (Fig. 5B). To our knowledge, this is the first report that Elk-1 activity is increased in colorectal cancer.

**Figure 4.** PGE2 induces activation of the Ras-MAPK cascade. A, HCA-7 cells were treated with PGE2 (0.1 μmol/L) for the indicated times after serum-starved for 24 hours. GTP-bound Ras was affinity-precipitated from 400 μg of whole cell extract using a GST-c-Raf-1 ras binding domain fusion protein. GTP-bound Ras proteins were detected by Western blotting using a pan-Ras antibody. B, amount of total Ras protein showing equal protein in each sample. Representative of three different experiments showing similar results. C, HCA-7 cells were serum-starved for 24 hours before PGE2 treatment at indicated concentrations of PGE2 for 5 and 180 minutes. Ras activation assays were done as mentioned above. Representative of three different experiments with similar results. C, PGE2 enhances activation of ERK and Elk-1. HCA-7 cells were treated with PGE2 at indicated concentrations for 5 and 180 minutes after serum-starved for 24 hours. ERK1/2 and Elk-1 activation was detected by measuring the levels of phosphorylated ERK1/2 and Elk-1 by Western blotting using anti-phospho-ERK1/2 (Tyr204) or anti-phospho-Elk-1 (Ser383) antibody, respectively. The blots were reprobed with ERK1/2 or Elk-1 antibody to monitor equal loading of samples. This figure is representative of three different experiments with similar results. D, effect of a Ras inhibitor (Fts-III) or MEK inhibitor (PD98059) on PGE2-induced ERK1/2 and Elk-1 activation. The cells were pretreated with the indicated inhibitors for 1 hour after serum starvation for 24 hours and then incubated with 0.1 μmol/L PGE2 for 5 minutes. ERK1/2 and Elk-1 activation was measured following the same procedure as mentioned above. Representative of three different experiments with similar results.
Taken together, these results show that PGE2 up-regulates COX-2 expression through activation of the Ras-MAPK pathway.

**A Ras-MAPK Cascade Is Required for PGE2-Stimulated Cell Proliferation.** Finally, we examined whether the Ras-MAPK pathway is required for PGE2-stimulated cell proliferation. HCA-7 cells were pretreated with either the Ras or MEK inhibitor for 1 hour, and then treated with indicated concentration of PGE2. Both cell proliferation by ELISA and cell counting were used to measure cell proliferation and cell growth. We observed that PGE2-induced cell proliferation was totally blocked by the Ras inhibitor at 20 μmol/L or MEK inhibitor at 25 μmol/L, respectively (Fig. 6). These data show that the Ras-MAPK cascade is required for PGE2-stimulated cell proliferation and growth.

**Discussion**

There is considerable preclinical evidence indicating that COX-2 plays a role in the development of various types of cancers, including colorectal, breast, bladder, skin and lung cancer. COX-2 derived PGE2 was found to promote colorectal adenoma growth in Apcmin mice. The aim of our current study was to understand the molecular mechanism(s) by which PGE2 promotes intestinal tumor growth.

Our laboratory was the first to report significant elevation of COX-2 expression in 85% of human colorectal carcinomas and in ~50% of colorectal adenomas (1). However, the mechanism(s) by which COX-2 is highly expressed in a number of solid malignancies is not yet completely understood. COX-2 mRNA and protein are normally very low or undetectable in normal intestinal tissues, but are rapidly induced in response to inflammation, cytokines, growth factors, oncogenes, endotoxins, and other chemicals. Here we show that PGE2 can amplify the expression of COX-2, a key regulatory enzyme in the PG biosynthetic pathway, in colorectal carcinoma cells through a positive feedback loop. This self-amplifying loop may help explain why COX-2 is constitutively overexpressed in majority of colorectal cancers.
Our results show that PGE2 activates Ras at early (5 minutes) and late times (3-6 hours). A likely explanation for this observation is that there is a negative feedback loop that inactivates EP receptor function following ligand binding, which in turn inhibits Ras activation. In general, ligand binding to G-protein coupled receptors results in receptor phosphorylation, desensitization, and sequestration. For example, chemokine receptor CXCR2 desensitization occurs within 1 minute, whereas receptor sequestration is a much later event (30-60 minutes) (40). We postulate that PGE2 binding to its cognate receptors also leads to the desensitization and sequestration of EP receptors, which provides negative feedback after 5 minutes of PGE2 stimulation (Fig. 4A). Another possible explanation is that PGE2 is metabolized to an inactive cyclopentenone PGA2 in cultures similar to that has been observed in other biological fluids (41). Therefore, Ras activation in the late activation phase may depend primarily upon newly synthesized PGE2 through an autocrine loop.

Our results also show that Ras activation during the early phase results in increased COX-2 expression via a MAPK-dependent pathway. We have previously shown that COX-2 expression is elevated within 30 minutes after transforming growth factor α or 12-O-tetradecanoylphorbol-13-acetate treatment in rat intestinal epithelial (RIE-1) cells (42). In addition, PGE2 increases COX-2 mRNA expression in human prostatic carcinoma PC-3 cells with the highest levels of stimulation seen at 3 hours (43). These and our present results suggest that the constitutive (late) Ras activation is dependent on the newly synthesized PGE2 by COX-2. Alternatively, increased levels of PGE synthesis in addition to COX-2 may drive new synthesis of PGE2. This is consistent with the observation that PGE synthase is up-regulated after activation of Ras in colorectal cancer cells. Role of mitogen-activated protein kinases. J Biol Chem 1996;271:518-23.

Acknowledgments

Received 10/13/2004; revised 12/17/2004; accepted 12/28/2004.


The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

23. Wang D, Wang H, Shi Q, et al. Prostaglandin E2 (PGE2) promotes colorectal adenoma growth via epidermal (RIE-1) cells (42). In addition, PGE2 increases COX-2 expression via a MAPK-dependent pathway. Thus, PGE2 may mimic the effects of an aberrant Ras function even in the absence of an actual Ras gene mutation. This implies that the influence of wild-type Ras in human colorectal cancer is even greater than what is expected based on the known frequency of Ras mutations.
Prostaglandin E$_2$ Enhances Intestinal Adenoma Growth via Activation of the Ras-Mitogen-Activated Protein Kinase Cascade

Dingzhi Wang, F. Gregory Buchanan, Haibin Wang, et al.