THE FIBROBLAST GROWTH FACTOR (FGF) family affects the proliferation, differentiation, and migration of intestinal epithelial cells in vitro (3, 5, 22, 45). In addition, members of this family are known to be associated with intestinal pathology, including inflammatory bowel disease and cancer (3, 5, 10, 22, 44, 45). The effect of FGFs on biological events involved in repair as well as the broad range of cell types that respond to these growth factors make them prime candidates as mediators of the intestinal response to injury (3, 4, 22, 27, 45). Indeed, one member of the FGF family, basic FGF (bFGF), has been shown to increase intestinal stem cell survival in the mouse after radiation injury, as measured by the number of regenerative crypts (18). The signaling events initiated by bFGF and how these downstream targets ultimately converge to affect stem cell survival remains unclear.

Prostaglandins are known modulators of the intestinal response to radiation injury. bFGF induces prostaglandin synthesis in a variety of cell types, often in the context of angiogenesis or tissue remodeling/healing (23, 26, 30). When a stable analog of PGE$_2$, dimethyl PGE$_2$, is given to mice before radiation, the number of surviving crypts is increased (15). Although dimethyl PGE$_2$ given alone after radiation does not change crypt survival, it does reverse the ability of indomethacin to suppress the number of crypts surviving radiation injury (6). Similarly, irradiated cyclooxygenase (COX)-1$^{-/-}$ mice show a reduced number of surviving crypts and an increased number of apoptotic cells compared with their wild-type littermates (19). Together, these data raise the possibility that bFGF modulates radiation injury through the induction of prostaglandins.

Much of the work regarding FGF signal transduction has centered on the proliferative effects of these growth factors and has emphasized the ERK signal-transduction module. Less is known regarding the ability of FGFs to interact with other signal-transduction modules such as p38 MAPK, Src, protein kinase C, phospholipase C, and phosphatidylinositol 3’ kinase. The relative contributions of activation of these different signaling pathways to the biological effects of bFGF are unknown. At least two FGF-induced effects, proliferation and migration, are activated through different signaling pathways (Src and p38, respectively) (4, 27). Most studies regarding the ability of bFGF to activate p38 have concentrated on endothelial cells and the role of p38 in migration and angiogenesis. Whether bFGF stimulation of intestinal epithelial cells results in activation of p38 and the downstream
consequences of this activation has not been explored previously.

The COX-2 gene is commonly upregulated by two general classes of mediators: growth factors and inflammatory cytokines. Depending on the stimulus and cell target, the NF-κB signaling pathway and/or any one of the MAPK modules may be involved in activating COX-2 expression (40). The COX-2 promoter contains a number of potential regulatory elements; one of these elements, the ATF/CRE site, binds transcription factor complexes whose formation can be modulated by activation of p38 (40). Although small molecule inhibitors of p38 have been used to demonstrate a requirement for p38 in upregulated COX-2 expression (8, 11, 12, 14, 21, 37, 41), the contribution of p38-dependent transcriptional activation to the observed increase in COX-2 expression has been explored less extensively. Posttranscriptional regulation also plays a significant role in induced COX-2 expression (7, 9, 16). Run-on experiments indicate that increased transcription accounts for only a small component of the observed increase in COX-2 mRNA in some cell types treated with inflammatory stimuli. For stimuli such as IL-1β, LPS, and TNF-α, message stabilization appears to be the primary mechanism in COX-2 induction (2, 20). Not surprisingly, the stress-activated MAPK, p38, appears to be required for this enhanced stability (8, 11, 12, 21, 29, 35). Message stabilization also contributes to the induced levels of COX-2 observed in “growth-stimulated” cells such as colon carcinomas (37), transforming growth factor (TGF)-β-stimulated (39), or ceramide and bile acid-treated ras-transformed rat intestinal cells (47), IFN-γ-stimulated keratinocytes (31), and nucleotide-stimulated (46) or angiotensin II (33)-treated smooth muscle cells. In most but not all of these instances, p38 also appears to play a role in COX-2 mRNA stability (31, 33, 47). The ability of bFGF to upregulate COX-2 through a p38-dependent mechanism has not previously been evaluated.

**MATERIALS AND METHODS**

**Cell culture and transfected cell lines.** The human intestinal cell line I407 (American Type Culture Collection; Manassas, VA) was maintained in basal medium Eagle with Earl’s salts (GIBCO-BRL; Grand Island, NY), supplemented with 6 mM glutamine, 10% heat-inactivated fetal calf serum, and antibiotics (penicillin 50 U/ml and streptomycin 50 μg/ml). Control-transfected, p38α, wild-type (p38αWT), and p38α dominant-negative (p38αDN) stable transfectant cell lines were maintained in the above media supplemented with G-418 as the antibiotic, but were cultured for 1 wk in the absence of G-418 before use in experiments. Briefly, 50–80% confluent cultures of I407 cells seeded in six-well tissue culture plates were transfected with FuGENE 6 reagent (Roche; Indianapolis, IN) according to the manufacturer’s guidelines at a ratio of 3:2 FuGENE/DNA and 2 μg DNA/well. pCMV-p38α wild-type and pCMV-p38α dominant-negative (Thr<sup>180</sup> and Tyr<sup>182</sup> replaced with Ala and Phe, respectively) expression plasmids used for transfection were obtained from Dr. R. Davis, Howard Hughes Medical Institute. pcDNA 3.1 (Invitrogen; Carlsbad, CA) was used as the empty vector for control transfections. After overnight exposure to the DNA/FuGENE complex, the media was changed, and two days later, cultures were placed into media containing 1.2 mg/ml G-418 for selection of stable transfected clones.

The human adenocarcinoma cell lines HT-29 and Caco-2 were grown in Dulbecco’s modified Eagle’s medium (BioWhittaker; Walkersville, MD) supplemented with 10% heat-inactivated fetal calf serum, antibiotics (as above), 10 mM nonessential amino acids, and an additional 200 mM glutamine.

**COX mRNA and protein levels.** The ability of bFGF to regulate COX-2 was determined by stimulating subconfluent I407 cells with bFGF (Scios-Nova; Mountainview, CA) at 10 ng/ml plus heparin at 10 μg/ml. Subconfluent HT-29 and Caco-2 cells were serum starved (0.1% fetal calf serum) overnight and then stimulated with bFGF (as for I407 cells) in media containing 0.1% fetal calf serum with or without the p38 inhibitor SB-203580 (10 μM). At the indicated times, cells were lysed with Laemmlí buffer for subsequent detection of COX-2 protein by Western analysis or lysed with a guandine thiocyanate-based solution (Ambion Direct Protect kit) for subsequent ribonuclease protection assays.

**Western analysis.** Lysates were separated by SDS-PAGE, blotted to Immobilon-P membrane (Millipore; Bedford, MA), and COX-2 protein detected using an anti-COX-2 antibody from Santa Cruz Biotechnology (Santa Cruz, CA) according to the protocol suggested by the supplier. Immunoreactive protein was detected by enhanced chemiluminescence (ECL) reagent (Amershams, Piscataway, NJ) and corrected to actin determined by reprobing the same blot (antibody from Santa Cruz). COX-1 protein expression was determined in the same manner using a COX-1 antibody from Santa Cruz.

COX-2 mRNA levels were evaluated using the Direct Protect ribonuclease protection kit from Ambion (Austin, TX). The 400-nucleotide antisense human COX-2 32P-labeled probe was generated using SauI linearized plasmid (gift of Dr. J. Masferrer, Pharmacia) and T7 polymerase (Maxiscript kit, Ambion). The 32P-labeled cyclophilin probe was generated using a template from Ambion. After gel purification of the probes, they were combined with the cell lysate, and the ribonuclease protection assay was performed as directed in the kit instructions. Protected fragments were separated by electrophoresis, and radioactivity was detected using autoradiography on Kodak Biomax MR film or using the PhosphorImager SI (Molecular Dynamics; Sunnyvale, CA). The COX-2 signal was corrected to cyclophilin. COX-1 mRNA levels were determined in a similar manner. For COX-1 cRNAs, a 240-nucleotide antisense human COX-1 32P-labeled probe was generated from an EcoRV-linearized plasmid from Dr. J. Masferrer (Pharmacia) using T7 polymerase.

**COX-2 mRNA stability.** For experiments determining the stability of COX-2 mRNA, COX-2 mRNA levels were evaluated using real-time RT-PCR. Cells were stimulated for 1 h with bFGF followed by the addition of dichlorobenzimidazole riboside (DRB; 100 μM) or DRB and the p38 inhibitor SB-203580 (10 μM) for the indicated times. RNA was isolated using TriZOL (Invitrogen) and reverse transcribed using Superscript II RT (Invitrogen) with random hexamers according to the manufacturer’s instructions. Real-time PCR was performed in an iCycler (Bio-Rad; Hercules, CA) using SYBR Green PCR master mix (Applied Biosystems). COX-2 primers (0.2 μM each) were forward: 5’-ATC CTA AAT GGT GTG ATG AG-3’; reverse: 5’-GCC ACT CAA GTG TTG CAC AT-3’. GAPDH primers (0.3 μM each) were forward: 5’-GAA GGT GAA GGT CGC AGT C-3’; reverse: 5’-GAA GGT GAT GGG ATT TC-3’. Reaction conditions were as follows: 10 min at 95°C, then 40 cycles of 95°C (15 s) and 60°C (60 s), with
data acquisition during the 1-min 60°C step. Melt analysis was used to confirm PCR products.

Transcriptional activation of the COX-2 promoter by bFGF. I407 cells were transfected with a human COX-2 promoter/luciferase reporter plasmid containing the region –1432/+59 of the human COX-2 promoter in the pGL3 reporter plasmid (generously supplied by Dr. T. Tanabe, National Cardiovascular Research Institute, Japan). Control vectors for promoter activity were pGL3-basic (negative control; Promega) and pGL3-control (positive control; Promega). pSV-β-galactosidase vector (Promega) was cotransfected as a transfection control. Subconfluent cells in 12-well plates were transfected using FuGENE 6 as suggested by the manufacturer at a ratio of 3:1 FuGENE 6 to DNA. Each well of cells received a total of 0.3 μg DNA (0.2 μg of the promoter reporter plasmid and 0.1 μg of the transfection control plasmid). Twenty-four hours later, the transfected cells were placed into fresh media with or without bFGF as described above. For experiments using the p38 MAPK inhibitor, SB-203580 (10 μM) was added 1 h before the media change and was included during the treatment period. At the indicated times after stimulation, cells were lysed, protein and β-galactosidase activity was assayed using kits from Promega.

As a positive control for the promoter activity of the human COX (hCOX)-2 plasmid, I407 cells were transfected as above with the pSV-β-galactosidase and hCOX-2 promoter/luciferase plasmids. Approximately 30 h after the start of transfection, cells were serum-starved overnight (media containing 0.1% fetal calf serum) and then incubated with various stimuli for the indicated times. Luciferase and β-galactosidase activities were then assayed as described above.

p38 Activation. To determine the time course of p38 activation, equivalent amounts of protein from bFGF-stimulated I407 cells were separated by SDS-PAGE, blotted to Immobilon-P, and probed with an antibody specific for the doubly phosphorylated form of p38 (Cell Signaling Technology; Beverly, MA) using the protocol suggested by the supplier.

PGE2 enzyme immunoassay. PGE2 levels were determined by analyzing the media from cells stimulated with bFGF using a kit from Cayman (Ann Arbor, MI) as described above. For time course experiments, media were collected every 12 h and replaced with fresh media. Inhibitors were added at the time of stimulation and at the time of media replacement.

In experiments testing the effect of exogenous arachidonate on PGE2 synthesis, cells were stimulated for either 2 or 24 h with bFGF; the media was then replaced with fresh media containing vehicle or 10 μM arachidonate and collected after 15 min.

PGE2 production. Whole cell lysates were prepared from I407 cells grown to various cell densities in 25-cm flasks. The monolayers were washed with PBS, 1 ml lysate buffer (N-Tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES); 20 mM), pH 7.4, 2 mM DTT, 10% glycerol, 25 μg/ml each antipain, aprotinin, leupeptin, and chymostatin, 50 μM phenanthroline, 10 μg/ml pepstatin A, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 200 μM sodium orthovanadate, 0.1 mM PMSF, and 1% Triton X-100 was pipetted onto each. The flasks were incubated for 30 min at 4°C, and lysates were collected into 1.5-ml tubes and cleared by spinning 15 min at 10,000 g at 4°C. The supernatants were transferred to a clean tube, then aliquotted and frozen at –20°C. Concentration was determined by the Bradford protein assay (Bio-Rad).

Proteins were resolved on polyacrylamide gels (4–15% gradient Ready Gels; Bio-Rad) and transferred to polyvinylidene difluoride membranes by electrophoretic transfer. The proteins were fixed and stained with 0.1% Coomassie blue in 10% acetic acid, 40% methanol to confirm that the proteins had transferred. They were rewet with methanol, reequilibrated with H2O, and blocked overnight in 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.05% Triton X-100 (TBST) with 5% dry milk at 4°C. The primary antibodies used to identify the receptor proteins were the anti-FGF receptors anti-FR-1 (C15; Santa Cruz), anti-FR-2 (C17; Santa Cruz), anti-FR-3 (C15; Santa Cruz), and anti-FR-4 (C16; Santa Cruz). The membranes were incubated for 1–1.5 h with primary antibody at 1 μg/ml in TBST, washed in TBST, and then incubated for 1 h at room temperature or overnight at 4°C with anti-rabbit IgG conjugated to horseradish peroxidase at the manufacturer’s recommended concentration. Bound antibodies were detected using ECL (Amer sham).

FGF receptor (FGFR) expression was confirmed using RT-PCR. Total RNA was isolated from subconfluent, growing cultures of I407 as well as Caco-2 cells using the RNeasy miniprep (Qiagen) and reverse transcribed using Superscript II RT (Invitrogen) with random hexamers according to the manufacturer’s instructions. Real-time PCR was performed with the SD-57000 (Applied Biosystems; Foster City, CA) using SYBR Green PCR master mix (Applied Biosystems). Primer pairs for each of the four receptors were designed using Primer Express software (Applied Biosystems) and were as follows: 1) FGFR1, forward: 5′-CAGGGGACATTCAC-ACACAC-3′, reverse: 5′-GGTGTCATCATCCACTACA-3′; 2) FGFR2, forward: 5′-AACGTTCAAGCAGTTGGTAGAAGAC-3′, reverse: 5′-CAGGTAACTAGGTGATCTTGCAGA-3′; 3) FGFR3, forward: 5′-ACGGCACACCCTACGTTACC-3′, reverse: 5′-TGGGAAACTGTTGCAGAATTCTG-3′; 4) FGFR4, forward: 5′-TGCGTAAAGCAGACCTGTCAT-3′, reverse: 5′-TCACCTCTGACCTTGTACACAT-3′. Primers were used each at 0.25 μM. Reaction conditions were as follows: 2 min at 50°C, then 1 cycle of 95°C (10 min) and 34 cycles of 95°C (15 s), then 60°C (60 s) with data acquisition during the 1-min 60°C step. Melt analysis was used to confirm PCR products. Subsequently, PCR products were resolved by electrophoresis in a 1% agarose gel and visualized by photographing under ultraviolet light using Polapan 667 film (Polaroid).

RESULTS

In our study of the regulation of COX-2 expression by bFGF, we first examined the expression of FGFRs in I407 cells by Western blot analysis (Fig. 1). Each of the FGFR antibodies used was directed toward an epitope on the COOH terminus of the receptor. FR-3 was easily detectable by Western blotting in both preconfluent (50%) and confluent cultures of I407 cells. Low levels of FR-1, -2, and -4 could also be detected on prolonged exposures of Western blots from both preconfluent and confluent cultures. There was no change in expression of any of the FGFRs with the degree of confluence of the cell cultures. RT-PCR indicated the presence of FR-1, -3, and -4 in I407 cells (Fig. 1E).

I407 cells stimulated with bFGF began synthesizing PGE2 within 12 h (Fig. 2). PGE2 production remained elevated for 36 h and was inhibited by both indomethacin, a nonselective COX inhibitor, and by NS-398, a selective COX-2 inhibitor (Fig. 2). Concomitant with increased PGE2 synthesis, bFGF-stimulated cells also rapidly upregulated COX-2 mRNA (Fig. 3A) and COX-2 protein (Fig. 3B). Neither COX-1 mRNA, nor protein levels were increased with bFGF treatment.
These data indicate that bFGF up-regulates PGE2 synthesis in I407 cells through a COX-2-dependent mechanism.

Treatment of I407 cells with bFGF induced phosphorylation of p38 MAPK within 5 min; p38 remained phosphorylated for 3 h. This time course is consistent with a role for p38 activation in bFGF-stimulated PGE2 synthesis (Fig. 4). We, therefore, examined the role of p38 activation in the events controlling PGE2 production by stimulated cells. The p38 inhibitor SB-203580 completely abrogated PGE2 synthesis in bFGF-treated cells (Fig. 5). The role of p38 in the events leading to PGE2 production by bFGF-stimulated cells was established using I407 cells stably transfected with either p38αWT, p38αDN, or empty vector (control transfected). Transfection with p38αWT did not increase basal PGE2 synthesis nor did it further enhance the ability of I407 cells to synthesize PGE2 after bFGF stimulation (Fig. 6A). However, I407 cells expressing p38αDN were unable to increase PGE2 synthesis in response to bFGF (Fig. 6A).

Western blot analysis of cell lysates revealed that in contrast to nontransfected, control-transfected, and p38αWT-transfected cells, cells transfected with p38αDN did not upregulate COX-2 protein expression following bFGF treatment (Fig. 6B). Thus induction of COX-2 by bFGF appears to require the α isoform of p38.

The failure of p38αDN cells to produce PGE2 and to upregulate COX-2 synthesis in response to bFGF suggests that bFGF-stimulated PGE2 synthesis requires COX-2 induction. However, it is possible that the observed levels of COX-2 are sufficient and that the defect in bFGF-induced PGE2 synthesis is at the level of arachidonate release. cPLA2 can be phosphorylated by p38, and phosphorylation is a critical event in cPLA2 activation (40). To investigate the possibility that the defect in bFGF-induced PGE2 synthesis in p38αDN cells was due to diminished PLA2 activation, we examined the effect of exogenous arachidonate on PGE2 synthesis in bFGF-stimulated cells. In resting control-transfected, nontransfected, p38αDN, and p38αWT cells, PGE2 synthesis was ~8 pg/ml (data not shown).
All cell types showed an approximate fourfold increase in PGE$_2$ production (~33 pg/ml) when supplied with exogenous arachidonate (data not shown). Two time points following bFGF stimulation were examined, an
early (2 h) time point at which time COX-2 protein levels are just beginning to increase (and therefore might be limiting) and a late (24 h) time point at which COX-2 protein levels have plateaued (and arachidonate release may be limiting). Exogenous arachidonate did not further increase PGE2 synthesis in any of the cells stimulated for 2 h with bFGF, indicating that at this time, the amount of PGE2 produced is limited by COX-2 protein (Fig. 7). However, exogenous arachidonate resulted in increased PGE2 synthesis by nontransfected, control-transfected, and p38αWT-transfected cells stimulated for 24 h with bFGF (Fig. 7). These data indicate that late after bFGF stimulation, the induced level of COX-2 enzyme activity is sufficient to metabolize all the available endogenous substrate; thus arachidonate release is the limiting factor in PGE2 synthesis 24 h after bFGF stimulation. At neither time point was exogenous arachidonate able to overcome the defect in PGE2 synthesis in p38αDN cells. Thus, in I407 cells, bFGF stimulates PGE2 synthesis through a p38-dependent mechanism that requires upregulation of COX-2 protein.

COX-2 protein levels may be regulated by both transcriptional and posttranscriptional mechanisms. We investigated the effect of bFGF on COX-2 transcription using a COX-2 promoter/luciferase reporter construct containing the region −1432/59 of the human COX-2 promoter. I407 cells transfected with the luciferase vector without a promoter (pGL3-basic) averaged 3.8 × 10⁴ relative light units (RLU) at the end of transfection, and cells transfected with the luciferase vector under the control of the SV40 promoter (pGL3-control) averaged 4.6 × 10⁵ RLU (data not shown). We found that the COX-2 promoter is active in unstimulated I407 cells (3.1 × 10⁶ RLU) and that promoter activity is not further increased by bFGF treatment (Fig. 8A). Treatment of transfected cells with SB-203580 (1 h before and during stimulation) did not alter COX-2 promoter activity (Fig. 8B). We confirmed our ability to detect changes in activity of the transfected hCOX-2 promoter/luciferase plasmid using various stimuli. As shown in Fig. 9, activity of the hCOX-2 promoter was

![Graph 1](image1)

**Fig. 6.** I407 cells stably transfected with p38α dominant-negative (DN) construct do not produce PGE2 or upregulate COX-2 after bFGF treatment. A: PGE2 levels were determined 24 h after addition of media or bFGF to nontransfected (NT), control-transfected (CT), p38αDN-transfected, or p38α wild-type (WT)-transfected I407 cells. Data are presented as the means ± SE of replicate wells assayed in duplicate and are representative of at least 2 separate experiments. B: COX-2 protein levels in similarly treated cells were analyzed by Western analysis. Data are the means ± SE of duplicate samples and are representative of at least 2 independent experiments.

![Graph 2](image2)

**Fig. 7.** Exogenous arachidonate does not restore PGE2 synthesis by bFGF-treated p38αDN stable transfectants. NT, CT, p38αDN-transfected, or p38αWT-transfected I407 cells were stimulated 2 or 24 h with bFGF. Fresh media containing either vehicle or 10 μM arachidonate was then placed on the cells and collected 15 min later for analysis of PGE2. Duplicate wells were assayed. Data are the means ± SE and are representative of at least 2 separate experiments.
increased when serum-starved cells were incubated with media containing 10% fetal calf serum. Similarly, serum-starved cells stimulated with 20 ng/ml IL-1β showed a detectable increase in promoter activity (Fig. 9). C2-ceramide (10 μM) did not stimulate promoter activity (data not shown).

We next assessed whether the observed p38-dependent net increase in COX-2 mRNA following bFGF stimulation was due to message stabilization. For these experiments, I407 cells were treated for 1 h with or without bFGF and then incubated with DRB with and without the p38 inhibitor SB-203580. In untreated I407 cells, COX-2 mRNA rapidly decayed, with a half-life (t1/2) of ~15 min and a 90% loss within the first 30 min after DRB addition (Fig. 10). In cells treated with bFGF, COX-2 mRNA stability was increased to a t1/2 of 60 min (Fig. 10). Inhibition of p38 with SB-203580 reduced the t1/2 of the COX-2 message from 60 min...

Fig. 8. COX-2 promoter activity in bFGF-treated I407 cells. I407 cells were transfected with a human COX-2 promoter/luciferase reporter plasmid containing the region −1432/+59 of the human COX-2 promoter in the pGL3 reporter plasmid along with the pSV-β-galactosidase vector. Twenty-four hours after transfection, cells were incubated with fresh media with or without bFGF. B: after transfection, cells were pretreated for 1 h with SB-203580 (10 μM) and then incubated with fresh media with or without bFGF containing 10 μM SB-203580. At the indicated times after stimulation, cells were lysed and luciferase and β-galactosidase activity was assayed. Data are the means of replicate wells ± SE and are representative of duplicate experiments.

Fig. 9. COX-2 promoter activity by serum-starved I407 cells. I407 cells were transfected with a human COX-2 promoter/luciferase reporter plasmid containing the region −1432/+59 of the human COX-2 promoter in the pGL3 reporter plasmid along with the pSV-β-galactosidase vector. Thirty hours after transfection, cells were serum starved overnight and then stimulated with fresh serum-starved media (starved), media containing 10% serum (serum), or IL-1β (20 ng/ml) in serum-starved media (IL-1β). At the indicated times after stimulation, cells were lysed and luciferase and β-galactosidase activity assayed. Data are the means of replicate wells ± SE and are representative of duplicate experiments.

Fig. 10. COX-2 mRNA stability is increased in bFGF-stimulated I407 cells and is attenuated by inhibition of p38. Cells were stimulated for 1 h with or without bFGF followed by addition of dichlorobenzimidazole riboside (DRB) or DRB + 10 μM SB203580 for the indicated times. The amount of COX-2 mRNA at each time point was determined by real-time PCR. Data are presented as the means of samples done in triplicate and are representative of at least 2 separate experiments done in duplicate.
observed in bFGF-stimulated cells to 30 min (Fig. 10). Thus the major mechanism responsible for increased COX-2 mRNA levels in bFGF-stimulated I407 cells is message stabilization, and p38 plays an important role in this stabilization.

bFGF-stimulated p38 MAPK-dependent COX-2 expression appears to be a general phenomenon in gastrointestinal epithelial cell lines, because bFGF also upregulated COX-2 expression in the colonic epithelial cell lines HT-29 and Caco-2 (Fig. 11). Both cell lines are reported to express receptors for bFGF (24, 32). bFGF increased COX-2 protein expression in both serum-starved HT-29 (Fig. 11A) and serum-starved Caco-2 (Fig. 11B) approximately twofold. These cell lines express high levels of COX-2 protein under normal growth conditions (10% fetal calf serum); therefore, the cells had to be serum-starved overnight to detect bFGF-induced upregulation. The p38 MAPK inhibitor SB-203580 attenuated bFGF-induced COX-2 protein expression by both cell lines (Fig. 11).

**DISCUSSION**

In this study, we have demonstrated that bFGF stimulates PGE2 synthesis in a human intestinal epithelial cell line via a p38α-dependent increase in COX-2 mRNA stability. In the absence of COX-2 upregulation, bFGF was unable to stimulate PGE2 synthesis in a p38αDN cell line even in the presence of exogenous arachidonate. Although bFGF has been demonstrated to affect epithelial stem cell survival in vivo after radiation injury (18), this is the first demonstration of a direct effect of bFGF on intestinal epithelial cells. Both HT-29 and Caco-2 cells have been reported to express receptors for bFGF (24, 32). Similar to I407 cells, we found that both of these cell lines increased COX-2 expression in response to bFGF and that the p38 MAPK inhibitor SB-203580 abrogated bFGF-induced COX-2 expression. These findings support the suggestion that the effects of bFGF on intestinal stem cell survival after radiation are direct effects on epithelial stem cells rather than being mediated by other cell types (34, 38).

COX-2 mRNA peaked within 1 h after bFGF treatment of I407 cells, with a subsequent decline to approximately threefold over basal at 24 h. COX-2 protein expression lagged slightly, with a maximal increase observed 3 h after bFGF. However, after the initial increase, COX-2 protein levels remained essentially stable up to 24 h. The relatively high levels of COX-2 protein at 24 h compared with the level of COX-2 mRNA may reflect the differences in COX-2 protein stability vs. COX-2 mRNA stability. Zhang et al. (47) observed a similar pattern in which COX-2 mRNA was near baseline 24 h after chenodeoxycholate treatment of rat intestinal epithelial cells, whereas COX-2 protein was still elevated. In an extensive comparison of COX-2 expression in colon carcinoma cell lines, Shao et al. (37) observed a good correlation between COX-2 mRNA and protein for some cell lines (e.g., HCA-7, Moser), whereas others appeared to have relatively low levels of message while expressing easily detectable protein (LS-174) or vice versa (HT-29). They also observed significant cell line-to-cell line variation in COX-2 protein stability (37).
The biological responses of bFGF are mediated through specific cell surface receptors that possess tyrosine kinase activity (3, 27). Binding of bFGF to its receptor activates a number of signaling pathways including phospholipase C, ERK-1, ERK-2, Src, and p38, which ultimately converge to elicit a particular biological effect (4, 27). Here, we demonstrate that the induction of COX-2 expression by bFGF in I407 cells is mediated through the α-isooform of p38. Although signaling through p38 has been associated with bFGF binding, this is the first association of bFGF with a specific p38 isoform. The induction of COX-2 expression by bFGF suggests the presence of one or more FGFRs on these cells. Western analysis indicated that I407 cells express FR-1, -2, -3, and -4, although FR-3 appeared to be most prominent. RT-PCR indicated the expression of FR-1, -3, and -4. This is consistent with previous reports of FR-3 expression in other intestinal epithelial cell lines including HT-29 and Caco-2 (24, 32).

Transcriptional regulation of COX-2 is mediated by a variety of cytokines and growth factors including transforming growth factor-α (TGF-α) (40). bFGF has been demonstrated to induce COX-2 expression in gastric epithelial cells (36), Syrian hamster embryo cells (1), osteoblasts (26), endothelial cells derived from bone (23), and aortic smooth muscle cells (25). In these studies, the only attempt to define the intracellular signaling involved in bFGF induction of COX-2 expression was the demonstration that PD-98059, an ERK-pathway inhibitor, inhibited bFGF-induced COX-2 expression in aortic smooth muscle cells (25). Here, we demonstrate that in intestinal epithelial cells, COX-2 induction is mediated through p38α, because I407 cells transfected with p38α-DN failed to upregulate PGE2 or COX-2 expression following bFGF treatment. However, I407 cells transfected with p38α-DN did not exhibit elevated basal levels of COX-2 or PGE2, nor did they exhibit any further increase in COX-2 expression or PGE2 synthesis following bFGF compared with nontransfected or control-transfected cells. These data are similar to the findings of Guan et al. (13), with rat primary mesangial cells stably transfected with similar constructs. However, transient transfection of a mammary epithelial cell line (41), human synovial fibroblasts (11), immortalized human articular chondrocytes (43), and the liver parenchymal-like cell line RL34 (28) with p38α-DN did not increase basal COX-2 promoter activity or protein expression. This difference in response may simply reflect experimental differences (i.e., different cell types, transient vs. stable transfection) or may reflect differences in the basal activity of upstream modulators of p38 activity.

PGE2 synthesis is dependent not only on the expression of COX, but also on the availability of arachidonic acid, the substrate for COX. bFGF is known to affect both COX-2 expression and arachidonate availability. In pancreatic acini, bFGF stimulates arachidonate release through sequential activation of tyrosine kinase, phospholipase C, protein kinase C, and diacylglycerol lipase (17). Unstimulated I407 cells produce ~8 pg/ml PGE2. When supplied with exogenous arachidonate, PGE2 synthesis increases approximately fourfold for nontransfected, control-transfected, p38α-WT, and p38α-DN cells, most likely reflecting metabolism by COX-1. Within 2 h after bFGF stimulation, control-transfected and nontransfected I407 cells exhibited an approximate 10-fold increase in the ability to synthesize PGE2, which was not further increased by exogenous arachidonate. These data suggest that bFGF-stimulated PGE2 synthesis occurs via increased metabolism by COX-2, and that endogenous levels of arachidonate are sufficient to saturate the cyclooxygenase present. After 24 h, exogenous arachidonate does increase PGE2 synthesis by nontransfected, control-transfected, and p38α-WT cells, indicating that, at this time, the ability to metabolize arachidonate is greater than the amount of endogenous substrate. In p38α-DN I407 cells, bFGF does not stimulate PGE2 production, even in the presence of exogenous arachidonate. This demonstrates that the p38α-mediated increase in PGE2 synthesis induced by bFGF requires COX-2 synthesis and is not mediated solely via arachidonate availability.

COX-2 mRNA levels are regulated both by the rate of transcription and by mRNA stability (16, 40). Which mechanism is dominant is a function of the stimulus; IL-1, for example, increases COX-2 mRNA levels primarily by enhancing mRNA stability (11, 20, 35). p38 Activation can affect mRNA levels either by increasing transcription or by stabilizing mRNA. In human mammary epithelial cells, taxol induces COX-2 through a p38-dependent mechanism; in this system, p38 increases COX-2 mRNA by enhancing transcription rather than by stabilizing mRNA (42). Our study demonstrates that bFGF increases COX-2 mRNA levels predominately by increasing COX-2 mRNA stability. In resting I407 cells, ~90% of the COX-2 message decays within 30 min. Similarly, Sheng et al. (39) found that COX-2 mRNA is short lived in rat intestinal epithelial cells (t1/2 = ~13 min). When ras expression in these cells was induced or when these cells were treated with TGF-β, COX-2 mRNA message was stabilized (t1/2 = ~30 min). When cells were induced to express ras and concomitantly treated with TGF-β, COX-2 mRNA t1/2 increased to ~1 h. In two colonic carcinoma cell lines with high basal COX-2 expression, LS-174 and HCA-7, COX-2 mRNA t1/2 was ~80 and ~120 min, respectively (37). The t1/2 of COX-2 mRNA in bFGF-stimulated cells is ~60 min and is reduced to ~30 min in the presence of the p38 inhibitor SB-203580. In HeLa cells treated with IL-1, COX-2 mRNA, t1/2 is ~60 min and is reduced to ~30 min in the presence of SB-203580 (35). Using a COX-2 promoter/luciferase construct, we found that in resting I407 cells, the COX-2 promoter is quite active and that no further increase in transcription from this promoter following bFGF treatment could be detected. However, stimuli, such as IL-1β and serum, previously demonstrated to increase COX-2 promoter activity in other cell lines (20, 40), did increase COX-2 promoter activity in serum-starved I407 cells. In I407 cells, IL-1β mod-
eratorily increased COX-2 transcriptional activity similar to the effect of IL-1β on COX-2 transcription in human macrophages (20). The increase in COX-2 message stability appears to be mediated through a p38α-dependent mechanism, because in the absence of p38α activation, bFGF fails to increase COX-2 expression and mRNA stability in bFGF-stimulated cells is attenuated by an inhibitor of p38.

bFGF mediates a variety of biological functions in injury repair, including stimulation of epithelial migration (3, 4, 22, 27) and enhanced stem cell survival after radiation (18). Here, we demonstrate that bFGF induces COX-2 in an intestinal epithelial cell line through a p38α-dependent mechanism. The ability of bFGF to induce COX-2 expression in a p38α-dependent manner was not confined to I407 cells. Both the HT-29 and Caco-2 gastrointestinal cell lines showed bFGF-induced increases in COX-2 protein expression were abrogated by the p38 inhibitor SB-203580. These data raise the possibility that the effects of bFGF observed in vivo, in the gut, may be due to a direct effect on the epithelial cells and that other biological effects of bFGF on the epithelium may also be mediated through p38α activation.

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