Regulation of Constitutive Cyclooxygenase-2 Expression in Colon Carcinoma Cells*

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Cyclooxygenase-2 (COX-2) is not normally expressed in the human large intestine, but its levels are increased in the majority of human colorectal carcinomas. Here we investigate the regulation of constitutive COX-2 expression and prostaglandin production in human colorectal carcinoma cells. Both COX-2 mRNA and protein were expressed in well differentiated HCA-7, Moser, LS-174, and HT-29 cells, albeit at different levels. COX-2 expression was not detected in several poorly differentiated colon cancer cell lines including DLD-1. Transcriptional regulation played a key role for the expression of COX-2 in human colon carcinoma cells, and both the nuclear factor for interleukin-6 regulatory element and the cAMP-response element were responsible for regulation of COX-2 transcription. COX-2 mRNA was more stable in HCA-7 cells than in the other cell lines tested. Both transcriptional and post-transcriptional regulation of COX-2 involved the MAP kinase pathway. Modulation of the Akt/protein kinase B or Rho B signaling pathways altered the levels of COX-2 expression. Furthermore, COX-2 protein is degraded through ubiquitin proteolysis, and its half-life was ∼3.5–8 h. HCA-7 cells produced significant quantities of prostaglandin E₂ and other prostaglandins. Moser and LS-174 cells also generated prostaglandins, but levels were significantly lower than that observed in HCA-7 cells.

Numerous studies have demonstrated a 40–50% reduction in the relative risk of colorectal cancer in individuals chronically taking nonsteroidal anti-inflammatory drugs compared with those not taking these agents (1–6). We have previously reported increased COX-2 expression in human colorectal adenocarcinomas compared with matched normal adjacent colonic mucosa (7). These findings have been confirmed by other studies that have shown elevated levels of COX-2 protein in colorectal tumors by Western blotting (8) and immunohistochemical staining (9–11). Additionally, lack of COX-2 expression results in decreased neoplastic growth and the number of tumors that develop in APC<sup>−/−</sup> mice (12). In several other animal models, treatment with selective COX-2 inhibitors results in a significant decrease in tumor growth (13–18). These observations suggest that COX-2 may play a critical role in colorectal carcinogenesis and represent an important target for prevention of colorectal cancer in humans.

Although increased expression of COX-2 in human and rodent intestinal tumors has been widely observed, the mechanisms that regulate the expression of COX-2 in colonic tumors are not completely understood. Transcriptional regulation of COX-2 has been evaluated in multiple cell lines (19). A number of studies have indicated that COX-2 expression is regulated at the transcriptional level in response to growth factors (20) and by signaling via oncogenic pathways in a variety of cells (21–24). Recent reports indicate that COX-2 is not only regulated at the level of transcription but also via post-transcriptional mechanisms. Interleukin-1α prolonged the half-life of the COX-2 mRNA, and dexamethasone treatment decreased COX-2 mRNA levels by post-transcriptional mechanisms (25, 26). We have reported that the induction of COX-2 in conditionally Ha-Ras<sup>Val-12</sup>-transformed Rat-1 cells occurs via a modest increase in COX-2 transcription with a significant increase in the stability of COX-2 mRNA (27). Induction of oncogenic Ras stabilizes the 3′-untranslated region (3′-UTR) of COX-2 mRNA in intestinal epithelial cells. A conserved AU-rich region (AU-rich element (ARE)) is responsible for the rapid turnover of COX-2 mRNA (28) and for the stabilization of COX-2 mRNA by Ras (29).

To further evaluate the molecular mechanisms that control the constitutive expression of COX-2 in human colon carcinoma cells, we sought to investigate the regulation of COX-2 at the level of transcription, post-transcription, and protein turnover. Our results demonstrate that expression of COX-2 is regulated at multiple levels, and this regulation involves a number of different signaling pathways.

EXPERIMENTAL PROCEDURES

Cell Culture—HCA-7 cells were a gift of Dr. Susan Kirkland (University of London). Moser, LS-174, HT-29, and DLD-1 cells were purchased from ATCC (Manassas, VA). The cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum. PD 98059 and LY 294002 were purchased from Calbiochem.

RNA Extraction and Northern Blot Analysis—The extraction of total cellular RNA was carried out as described previously (30). RNA samples (20 µg/lane) were separated on formaldehyde-agarose gels and blotted onto nitrocellulose membranes. The blots were hybridized with cDNA...
probes labeled with [32P]dCTP by random primer extension (Stratagene, La Jolla, CA). After hybridization and washes, the blots were subjected to autoradiography. 28 S rRNA signals were used as controls to determine the integrity of RNA and equality of loading in each lane. 

**Immunoblot Analysis**—Immunoblot analysis was performed as described previously (20). Briefly, the cells were lysed for 20 min in radioimmunoprecipitation assay buffer (1× phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 mM sodium orthovanadate). Clarified cell lysates were denatured and fractionated by SDS-polyacrylamide gel electrophoresis. After electrophoresis, the proteins were transferred to nitrocellulose membrane. The filters were then incubated with the indicated antibodies and developed by the enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech). Band density was quantitated by densitometry scanning. The anti-COX-2 antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**Nuclear Run-on Transcription Assay**—Nuclear run-on assays were performed as described previously (27). Nuclei were isolated from HCA-7, LS-174, and DLD-1 cells. In vitro run-on transcription was carried out by using 1 × 105 nuclei and 200 μCi of [32P]UTP/assay at 30 °C for 45 min. Labeled transcripts were purified by trichloroacetic acid precipitation. A total of 1 × 106 cpm of elongated nascent RNA per assay was hybridized for 48 h at 43 °C to filter-immobilized plasmid DNA. Filters were washed with 2× SSC at 45 °C for 2 h and incubated with RNase A (5 μg/ml) for 30 min at 37 °C and then for 1 h in 2× SSC. The autoradiograms were subjected to densitometric analysis, and all data were normalized to the internal control.

**Transfection of Reporter Constructs**—The assays to determine the activity of the COX-2 promoter and stability of COX-2 3′-UTR were described previously (29). Reporter constructs pHBS2 and -1432/ +59, -327/+59, -220/+59, -124/+59, -52/+59, CRM, ILM, and CRM-ILM) containing the 5′-flanking region of the human COX-2 gene were described previously (21). The reporter expression vectors, pLuc + 3′-UTR and pLuc + ARE were provided by Dr. Dan Dixon (University of Utah, Salt Lake City, UT) (28, 29). For transient transfections, cells were plated in 24-well plates 24 h prior to transfection. The cells were co-transfected with 0.5 μg of one of the COX-2 firefly luciferase plasmid constructs and 0.01 μg of the pRL-CMV plasmid, containing the Renilla luciferase gene (Promega, Madison WI), using the Lipofectin procedure (Life Technologies, Inc.) as described in the manufacturer’s protocol. Transfected cells were cultured for 24 h and lysed. 20 μl of lysate was used for both the firefly and Renilla luciferase readings. Firefly and Renilla luciferase activities were measured using a Dual-Luciferase Reporter assay system (Promega, Madison, WI) and a model TD-20/20 Luminometer. Firefly luciferase values were standardized to Renilla values.

The expression vectors containing the myristoylated form of Akt (Akt-myr) or dominant negative Akt-K179M cDNA were provided by Dr. Philip Tsichlis (Thomas Jefferson University, Philadelphia, PA). The expression vectors containing wild type Rho B, active Rho B (Rho B V14), and dominant negative Rho B (Rho B N19) were provided by Dr. George Prendergast (The Wistar Institute, Philadelphia, PA).

**Metabolic Labeling**—For these experiments, subconfluent cultures were established. Cells were incubated in methionine-free Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 200 μCi/ml of [35S]methionine (Amersham Pharmacia Biotech) for 3 h and then washed three times with Dulbecco’s modified Eagle’s medium. Fresh Dulbecco’s modified Eagle’s medium containing 2 mM unlabeled methionine was added to the culture medium. Cell lysates were collected in radioimmunoprecipitation assay buffer at the indicated time points. An equal amount of protein from each sample was immunoprecipitated with anti-COX-2 antibody (Santa Cruz Biotechnology), resolved by SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography.

**Quantitation of Eicosanoids**—For these experiments, subconfluent cell cultures were established. Fresh medium with 10% fetal bovine serum was replaced 24 h prior to harvesting the conditioned media. After collecting the culture media, serum-free medium supplemented with arachidonic acid (15 μM) (Cayman Chemical, Ann Arbor, MI) was added to the cells. The conditioned media was harvested after 1 h incubation with prostaglandin E2 (PGE2) levels in both serum-containing and serum-free media were quantitated by utilizing stable isotope dilution techniques employing gas chromatography negative ion chemical ionization mass spectrometry (31). The results are expressed as nanograms of PGE2 per 106 cells.

**Statistics**—All statistical analyses were performed by Student’s t test. The limit of significance for all tests was p < 0.05.

**RESULTS**

**COX-2 Expression in Colon Carcinoma Cells**—Although COX-2 is expressed in 80–90% of human colon carcinoma specimens, constitutive expression of COX-2 was only detected in a relatively small number of established colorectal carcinoma cell lines. We screened a number of available colon carcinoma cell lines and found that COX-2 was not detected in most of the poorly differentiated cell lines, including HCT 116, HCT 15, SW 480, SW 620, RKO, and DLD-1 cells but was expressed in many well differentiated cell lines (data not shown and Fig. 1).

Both COX-2 mRNA and protein were expressed in HCA-7, Moser, LS-174, and HT-29 cells. In contrast, no COX-2 expression was detected in DLD-1 cells (Fig. 1, A and B). The level of COX-2 mRNA in HCA-7 cells was 1.7-fold of that seen in Moser cells and about 10-fold the level seen in LS-174 or HT-29 cells. The highest level of COX-2 protein was observed in HCA-7 cells followed by the Moser and LS-174 cells. HT-29 cells expressed the lowest level of COX-2 protein among the COX-2-expressing cell lines.

**Transcriptional and Post-transcriptional Regulation of COX-2**—COX-2 transcription is regulated in response to a variety of growth factors and following activation of oncoproteins such as ras and src. In order to determine the mechanisms by which COX-2 expression is regulated in colon carcinoma cells, we compared the transcriptional activity of COX-2 in HCA-7, LS-174, and DLD-1 cells using nuclear run-on assays. The transcriptional activity of the COX-2 gene in HCA-7 cells was 3-fold higher than that seen in LS-174 cells, and almost no COX-2 transcription was detected in DLD-1 cells (Fig. 2A). In contrast, the transcriptional activity of a cell cycle protein, cyclin D1, was higher in the LS-174 cells. To determine the cis-acting elements that are responsible for the COX-2 transcription, a series of human COX-2 promoter deletion constructs were transfected in to HCA-7 and LS-174 cells. The highest transcriptional activity was achieved when the −220/+59 promoter construct was used. Removal of the NF-IL6 site (−124/+59) reduced the transcriptional activity by 68 and 45% in HCA-7 and LS-174 cells, respectively. Deletion of both the NF-IL6 and CRE (−52/+59) elements resulted in a >90% decrease in transcriptional activity of COX-2 (Fig. 2B). Mutating the CRE or NF-IL6 site in the COX-2 promoter region (−327/+59) reduced the transcriptional activity by 50–70%, and the promoter containing both mutated CRE and NF-IL6 element exhibited extremely low activity, similar to that seen with the −52/+59 construct.

Post-transcriptional regulation via stabilization of COX-2 mRNA plays an important role in the regulation of COX-2 in a variety of cultured cells. The stability of COX-2 mRNA was determined by the steady-state levels of COX-2 mRNA after transcription was blocked by the treatment with DRB (dichlorobenzimidazole riboside). The t1/2 of COX-2 mRNA was ~80 min in LS-174 cells and ~120 min in HCA-7 cells (Fig. 3A). Transient expression of the CMV promoter-driven luciferase reporter gene linked with 1.4 kilobase pairs of COX-2 3′-UTR (pLuc + 3′-UTR) revealed that the COX-2 3′-UTR was more
stable in HCA-7 cells than in LS-174 cells (Fig. 3B). Using the luciferase reporter vector containing the conserved AREs of the COX-2 3′-UTR demonstrated that the AREs found in the COX-2 3′-UTR were responsible for the difference in the stability of COX-2 mRNA between HCA-7 and LS-174 cells (Fig. 3C).

**Signaling Pathways Involved in Regulation of COX-2**—The mitogen-activated protein kinase pathway is important in both the transcriptional and post-transcriptional regulation of COX-2 expression. Inhibition of the activity of extracellular signal-regulated kinase (ERK) by the treatment with a specific MEK (mitogen-activated protein kinase/ERK kinase) inhibitor, PD 98059 (50 μM), reduced the COX-2 promoter activity by 35% in HCA-7 and LS-174 cells (Fig. 4, A and B). PD 98059 treatment also reduced the stability of COX-2 3′-UTR by 55% in these cells.

Akt/PKB can be modulated by multiple signaling pathways and acts as a transducer of many signals initiated by growth factor receptors that activate phosphatidylinositol 3-kinase (32). Cumulative evidence indicates that the phosphatidylinositol 3-kinase-Akt/PKB pathway is oncogenic and involved in the neoplastic transformation of mammalian cells. Therefore, it is of interest to determine whether Akt activity regulates the expression of COX-2. Co-transfection of a COX-2 promoter construct (pLuc suic/−1432/59) with a dominant negative Akt expression vector (Akt-K179M) slightly reduced the COX-2 promoter activity in LS-174 cells (Fig. 5A). However, co-transfection of COX-2 3′-UTR construct (pLuc suic/3′-UTR) with a constitutively active Akt expression vector (Akt-myr) resulted in 47 and 31% increase in the stability of COX-2 3′-UTR in HCA-7 and LS-174 cells (Fig. 5B). In contrast, expression of dominant negative Akt-K179M reduced the stability of COX-2 3′-UTR by 30% in HCA-7 cells.

The Rho family of proteins are key components in cellular processes that regulate gene expression, proliferation, motility, and invasion and are critical components in oncogenic Ras transformation (33). In order to determine whether Rho activity was involved in the regulation of COX-2, we co-transfected the Rho B expression vectors along with the constructs containing the COX-2 promoter or COX-2 3′-UTR. Transient expression of dominant negative Rho B N19 inhibited the activity of the COX-2 promoter by 42% in HCA-7 cells and by 37% in LS-174 cells (Fig. 5C), while expression of active Rho B V14 resulted in a 2.5-fold increase in the transcriptional activity of COX-2 in HCA-7 and a 2.3-fold increase in LS-174 cells. In contrast, the Rho B signaling pathway exerted less effect on the stability of COX-2 mRNA. Transiently expressing dominant negative Rho B N19 or active Rho B V14 resulted in less than 27% change of COX-2 3′-UTR stability in LS-174 cells and had almost no effect in HCA-7 cells (Fig. 5D).

To confirm the findings from the transient transfection experiments, HCA-7 and LS-174 cells were stably transfected with Akt and Rho B expression constructs. The levels of COX-2 protein were determined by Western analysis, and the results are shown in Fig. 6, A and B. Ectopic expression of Akt-K179M reduced COX-2 expression in both HCA-7 and LS-174 cells, while expression of active Akt-myr increased the levels of COX-2. Stable transfection with Rho B N19 decreased the levels of COX-2 in both HCA-7 and LS-174 cells, and expression of active Rho B V14 elevated the COX-2 expression. Therefore, regulation of COX-2 via modulation of Akt/PKB and Rho sig-
The mean sis revealed that treatment with MG-132 (10 carcinoma cells with a proteasome inhibitor, MG-132, and involved in the degradation of COX-2 protein, we treated colon way is involved in the degradation of many important cellular and HT-29 cells. We found that PGE2 is the predominant able. To estimate the activity of COX-2, we first determined the m 98059 (50 m) were co-transfected with phPES2-(−1472/+59) and pRL-CMV. PD 98059 (50 m) was added 24 h prior to harvest. Firefly luciferase values were standardized to Renilla values and compared with the paired controls. PD 98059 treatment had less than 10% effect on pRL-CMV luciferase. The mean ± S.E. of assays performed in quadruplicate are plotted, *, p < 0.05 compared with Me2SO-treated cells. The results were similar in three independent experiments. B, regulation of the stability of COX-2 3′-UTR by PD 98059. HCA-7 or LS-174 cells were co-transfected with pLuc + 3′-UTR and pRL-CMV plasmids. PD 98059 (50 m) was added 24 h prior to harvest. Firefly luciferase values were standardized to Renilla values and compared with the paired controls. The mean ± S.E. of assays performed in quadruplicate are plotted. *, p < 0.01 compared with Me2SO-treated cells. The results were similar in three independent experiments. RLU, relative luciferase units.

**FIG.4.** Regulation of COX-2 expression by PD 98059. A, regulation of COX-2 promoter activity by PD 98059. HCA-7 or LS-174 cells were co-transfected with phPES2-(−1472/+59) and pRL-CMV. PD 98059 (50 m) resulted in a rapid increase in the levels of COX-2 protein in HCA-7, Moser, LS-174, and HT-29 cells (Fig. 7A), indicating that COX-2 protein was degraded via the proteasome pathway in all of these cell lines. In response to MG-132 treatment, COX-2 protein levels were affected most in Moser and HT-29 cells and less in HCA-7 and LS-174 cells, suggesting that the activity of ubiquitin proteolysis may vary from cell line to cell line. To determine the stability of COX-2 protein, metabolic labeling experiments were performed in HCA-7, LS-174, Moser, and HT-29 cells. As shown in Fig. 7B, the t1/2 of COX-2 protein ranged from −3.5 h in Moser cells to −8 h in LS-174 cells. The stability of COX-2 protein varied from cell line to cell line.

**FIG.5.** Regulation of COX-2 expression by Akt/PKB and Rho B. A, regulation of COX-2 promoter activity by Akt. HCA-7 or LS-174 cells were co-transfected with phPES2-(−1472/+59), dominant negative Akt construct (pCMV-Akt-R178K), or constitutively active Akt (pCMV-Akt-myr) and pRL-CMV plasmid. After the cells were incubated for 24 h, firefly luciferase values were measured, standardized to Renilla values, and compared with empty vector-transfected cells. The means ± S.E. of assays performed in quadruplicate are plotted. *, p < 0.01 compared with empty vector-transfected cells. The results were similar in five independent experiments. B, regulation of the stability of COX-2 mRNA by Akt. HCA-7 or LS-174 cells were co-transfected with pLuc + 3′-UTR, pCMV-Akt-R178K, or pCMV-Akt-myr and pRL-CMV plasmid. After the cells were incubated for 24 h, firefly luciferase values were measured, standardized to Renilla values, and compared with empty vector-transfected cells. The means ± S.E. of assays performed in quadruplicate are plotted. *, p < 0.01 compared with empty vector-transfected cells. The results were similar in five independent experiments. C, regulation of COX-2 promoter activity by Rho B. HCA-7 or LS-174 cells were co-transfected with phPES2-(−1472/+59), Rho B expression vector (wild type Rho B, dominant negative Rho B N19, or constitutively active Rho B V14), and pRL-CMV plasmid. After the cells were incubated for 24 h, firefly luciferase values were measured, standardized to Renilla values, and compared with empty vector-transfected cells. The means ± S.E. of assays performed in quadruplicate are plotted. *, p < 0.01 compared with empty vector-transfected cells. The results were similar in four independent experiments. **DISCUSSION**

Prostaglandins derived from COX-2 are thought to play an important role in intestinal carcinogenesis, although the precise mechanism(s) by which they act is not yet understood. Understanding the pathways that control the expression of COX-2 and exploring the signaling mechanisms involved in the regulation of COX-2 expression may lead to a better understanding of its dysregulation in colorectal carcinomas. A number of studies have shown that COX-2 is expressed constitutively in the majority of colorectal carcinomas (7–10). COX-2 is not detectable in the normal colon of several different species of animals (8). In the present study, we found that COX-2 is constitutively expressed in the minority of established colon carcinoma cell lines. We demonstrate that four human colon cancer cell lines express both COX-2 mRNA and protein, in-
COX-2 protein. Similar results were observed in three separate experiments.

FIG. 6. Ectopic expression of Akt or Rho B. A, HCA-7 cells were transfected with the indicated Akt or Rho B expression constructs. Stable clones were selected by the treatment with zeocin and were pooled. The levels of COX-2 protein were compared by Western blot analysis. B, LS-174 cells were transfected with the indicated Akt or Rho B expression constructs. Stable clones were selected by the treatment with zeocin and were pooled. The levels of COX-2 were compared by Western blot analysis.

including the HCA-7, Moser, LS-174, and HT-29 cells. All of these cell lines were derived from well differentiated adenocarcinomas. These results are in agreement with the observation made in human carcinoma tissue (34). Expression of COX-2 mRNA has been reported in some undifferentiated colon carcinoma cell lines, including the HCT 116 cells (19). However, COX-2 mRNA is expressed at extremely low levels in HCT 116 cells, and COX-2 protein could not be detected (15, 35). The significance of constitutive COX-2 expression and prostaglandin synthesis in these well differentiated cell lines is not completely understood. There is mounting evidence that COX-2 expression in colorectal carcinoma cells provides a growth and survival advantage (35) and increases tumor cell invasiveness (36). Prostaglandin synthesis generated by these cells may provide a more suitable environment for cell growth and survival (35, 37).

COX-2 expression is regulated at both the transcriptional (21–24) and post-transcriptional levels (26–29). Our data suggest that transcriptional regulation plays the decisive role in the regulation of COX-2 expression in human colon carcinoma cells. Consistent with previous reports (21–24, 38, 39) the NF-IL6 site and CRE element are responsible for the transcriptional regulation of COX-2 in human colon cancer cells. We also found that the stability of COX-2 mRNA, in part, accounts for the steady-state levels of COX-2 mRNA in colon carcinoma cells that constitutively express COX-2. COX-2 mRNA stability appears to be regulated mainly via the conserved 116-nucleotide ARE.

COX-2 expression is not only regulated at the transcriptional and post-transcriptional levels but may also be regulated by the rate of protein synthesis and/or degradation. Dixon et al. (28) recently reported that the conserved 116-nucleotide ARE suppresses the translation of COX-2 mRNA into protein. The ubiquitin-proteasome pathway is the principle mechanism responsible for the turnover of short lived proteins in eukaryotic cells and is a fundamental mechanism for cellular control (reviewed in Ref. 40). A number of cellular regulatory proteins, including many cell cycle proteins, transcription factors, and growth factor receptors, are degraded by ubiquitin-proteasome-mediated proteolysis (reviewed in Ref. 41). COX-2 is the inducible isoform of cyclooxygenase and is not normally expressed under basal conditions but can be rapidly induced by growth factors, cytokines, and tumor promoters (20–24). Our results show that COX-2 protein is degraded via the proteasome pathway and that the stability of COX-2 protein varies from cell line to cell line.

Several signaling pathways have been implicated in the regulation of COX-2 expression. Activation of protein kinase C is responsible for the induction of COX-2 transcription by dihydroxy bile acids (42). Induction of COX-2 by the oncogene v- src (22) and platelet-derived growth factor or serum (20) requires activation of both Ras/Rac1/MEKK1/Jun N-terminal kinase and Ras/Raf-1/ERK signal transduction pathways. ERK, Jun N-terminal kinase, and p38 are required for the ceramide-induced expression of COX-2 (43). Activation of the p38 mitogen-activated protein kinase pathway induces the expression of COX-2 and increases prostaglandin biosynthesis (44). The p38 pathway has also been implicated in the control of COX-2 mRNA stability (45). We found that inhibition of MEK/ERK activity completely abolishes Ras-induced stabilization of COX-2 mRNA (27). In the present study, we demonstrate the importance of the MEK/ERK pathway for the constitutive expression of COX-2 in colorectal carcinoma cells. We also show that the Akt/PKB pathway and the Rho pathway can modulate COX-2 transcription and COX-2 mRNA stability. Interestingly, Rho B activity predominately regulates COX-2 expression at transcriptional level, and the Akt pathway predominantly modulates the stability of COX-2 mRNA. Akt/PKB regulates gene transcription by direct phosphorylation of transcription factors such as FKHR, FKHRL, and AFX (46–48) or by indirectly modifying CRE-binding protein (49, 50), E2F (51), or NF-κB (52). Evidence suggests that the Akt/PKB pathway promotes growth factor-mediated cell survival and inhibits apoptosis (53) via modifying the antiapoptotic and proapoptotic transcription factors such as FKHR, FKHRL, and AFX (46–48) or by indirectly modifying CRE-binding protein (49, 50), E2F (51), or NF-κB (52).
activities of members of the Bcl-2 gene family (54, 55). Members of the Rho family are key regulators of many cellular processes, such as the organization of the actin cytoskeleton, activation of kinase cascades, and regulation of gene expression, and they have been implicated as critical mediators for the transforming potential of diverse oncogenic pathways (reviewed in Ref. 33). Our results indicate that COX-2 is a target gene of the Akt/PKB and Rho B pathways and suggest that COX-2 may represent a downstream mediator of these oncogenic pathways.

COX-2 catalyzes the conversion of arachidonic acid to prostaglandin H₂ that is subsequently converted to a variety of eicosanoids including PGE₂, prostaglandin D₂, prostaglandin F₂α, prostaglandin I₂, and thromboxane A₂ (reviewed in Ref. 56). Although the COX-2 enzyme is constitutively expressed in HCA-7, Moser, LS-174, and HT-29 cells, PGE₂ was only detected in the HCA-7, Moser, and LS-174 cells. Treatment with celecoxib (3 μM) completely inhibited PGE₂ production in HCA-7 and Moser cells. These results suggest that selective COX-2 inhibitors might suppress the growth of HCA-7 (15) and Moser cells (16) through a COX-2-prostaglandin-dependent mechanism. Selective COX-2 inhibitors can also inhibit the growth of colon carcinoma cells in a COX-2-prostaglandin-dependent manner (reviewed in Ref. 57) when present in concentrations higher than those required to inhibit COX-2 enzyme activity.

In summary, our data indicate that constitutive expression of COX-2 in human colorectal carcinoma cells is regulated at the transcriptional, post-transcriptional, and protein levels. Prostaglandin formation correlates, to a significant extent, with COX-2 expression. A number of signaling pathways are involved in the regulation of COX-2 expression in colorectal carcinoma cells, and we find that the regulation of COX-2 expression is linked to key oncogenic signaling pathways such as Akt/PKB and Rho B.

REFERENCES

1. Thun, M. J., Namboodiri, M. M., and Heath, C. W. J. (1991) N. Engl. J. Med. 325, 1593–1596
2. Thun, M. J., Namboodiri, M. M., Calle, E. E., Flanders, W. D., and Heath, C. W. J. (1993) Cancer Res. 53, 1322–1327
3. Giovannucci, E., Willett, W. C. (1998) Ann. Intern. Med. 121, 241–246
4. Giovannucci, E., Willett, W. C. (1998) N. Engl. J. Med. 333, 609–614
5. Greenberg, E. R., Baron, J. A., Freeman, D. H. J., Mandel, J. S., and Haile, R. W. (1998) Cancer Res. 58, 362–366
6. Sheng, H., Williams, C. S., Shao, J., Liang, P., DuBois, R. N., and Beauchamp, R. D. (1996) J. Biol. Chem. 271, 31742–31748
7. Kandel, E. S., and Hay, N. (1999) Cell 107, 210–229
8. Matsuura, H., Matsuura, H., Hara, S., Yokoyama, C., and Tanabe, T. (1994) FASEB J. 8, 394–399
9. Hashimoto, J., Namboodiri, M. M., Heath, C. W. J., and Yabumoto, K. (1991) Cancer Res. 51, 5634–5637
10. Reddy, S. T., Wadleigh, D. J., and Herschman, H. R. (2000) J. Biol. Chem. 275, 11750–11757
11. Chapple, K. S., Cartwright, E. J., Hawcroft, G., Tisbury, A., Bonifer, C., Scott, N., Acker, W. J., and Prescott, S. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 325–331
12. Oshima, M., Dinchuk, J. E., Kargman, S., Oshima, H., Hancock, B., Kwong, E., and Jothy, S. (1999) Nat. Immunol. 1, 33956–33961.