Regulation of Cyclooxygenase-2 Expression by the Wnt and Ras Pathways

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ABSTRACT

Mutations in the adenomatous polyposis coli (APC) gene and K-ras occur in the majority of human colorectal cancers. Loss of functional APC protein activates the Wnt signal transduction pathway, allowing the nuclear accumulation of β-catenin, which then binds to T-cell factor-4 (Tcf-4), causing increased transcriptional activation of downstream target genes. We investigated the hypothesis that the activation of the Wnt pathway regulates cyclooxygenase-2 (COX-2). COX-2 was down-regulated after the induction of full-length APC in the HT29-APC cell line. We identified a Tcf-4-binding element (TBE) in the COX-2 promoter that specifically bound to Tcf-4 in an electrophoretic mobility shift assay. COX-2 promoter luciferase activity is down-regulated by APC in a promoter reporter construct containing the, but not with mutant TBE. Mutant β-catenin expression up-regulated the COX-2 promoter activity and the endogenous COX-2 mRNA expression in HuH7, hepatocellular carcinoma cell line, which is partially abrogated by cotransfection with a dominant-negative Tcf-4 expression vector. Although β-catenin alone did not increase COX-2 protein to detectable levels in HuH7 cells, coexpression of both mutant β-catenin and mutant K-ras increased COX-2 protein expression, which is consistent with the previous reports that K-ras can stabilize COX-2 mRNA. Taken together, our data support the hypothesis that COX-2 is down-regulated by APC and up-regulated by nuclear β-catenin accumulation, and additionally implicate the Wnt signal transduction pathway in colon and liver carcinogenesis.

INTRODUCTION

COX-2 is a regulatory factor in arachidonate metabolism. COX-2 expression is normally undetectable in most normal tissues, but can be induced rapidly during an inflammatory reaction (1, 2). COX-2 expression has been found to be associated with regulating cellular proliferation, differentiation, and tumorigenesis (2–4). Previous studies have shown that COX-2 participates in the colorectal carcinogenesis pathway, especially when APC mutation is the initiating event (2, 5). Direct genetic evidence that COX-2 plays a key role in colorectal tumorigenesis was provided by Oshima et al. (6), who showed that knocking out the COX-2 gene caused a marked reduction in the number and size of intestinal polyps in Apc knockout mice, a murine model of familial adenomatous polyposis. Nonsteroidal anti-inflammatory drugs dramatically reduce the incidence of polyps in Min mice and familial adenomatous polyposis patients (6, 7), both of which carry a germline APC mutation. APC gene inactivation plays a critical role at an early stage in the development of both inherited and sporadic forms of colorectal cancer (8, 9), also, mutant APC DNA has been detected in feces from patients with this cancer (10). APC is a member of the Wnt signal transduction pathway (11) and interacts with a variety of cytoplasmic proteins including glycogen synthase kinase-3β (12) axin family proteins (13), and β-catenin (14). Wild-type APC can bind to β-catenin and direct its intracellular degradation. However, mutated APC does not bind to β-catenin, resulting in its nuclear translocation (15). β-catenin is a major component of adherence junctions linking the actin cytoskeleton to members of the cadherin family of transmembrane cell-cell adhesion receptors. β-Catenin translocates into the nucleus, where it complexes with Tcf-4 transcription factors and regulates the expression of specific genes, e.g., c-myc, cyclin D1, and PPARα (16–20). Wnt family members are critical in developmental processes and have been shown to promote carcinogenesis (11). The Wnt-signaling pathway inactivates GSKin3β, which results in subsequent stabilization of β-catenin and stimulates Tcf-4-mediated gene transcription (21). Although the APC status affects COX-2 expression, and COX-2 has been proposed to be a downstream target of the Wnt-signaling pathway (5, 22–25), this hypothesis has not been fully investigated. In addition, the ras-signaling pathway has been shown to up-regulate COX-2 expression at its transcriptional and post-transcriptional level (26–28). In this study, we are testing the hypothesis that COX-2 is regulated by APC and β-catenin, and also ras signal transduction pathways.

MATERIALS AND METHODS

Cell Culture. The human colorectal cancer cell lines, HT29-APC and HT29-GAL, were generous gifts from Bert Vogelstein, The Johns Hopkins University Medical Institutions, Baltimore, MD (29). HT29, SW480, and HuH7 were obtained from American Type Culture Collection (Rockville, MD). The HCA7 cell line was kindly given by Susan Kirkland, Imperial College School of Medicine, London, UK (30). For culture of the HT29-APC cells, 0.6 mg/ml of hygromycin (Roche, Indianapolis, IN) was added. To induce full-length APC, HT29-APC cells were incubated with 100 μM of zinc chloride (Sigma, St. Louis, MO).

Plasmids. COX-2 promoter-luciferase constructs were generous gifts from Ray DuBois, Vanderbilt University Medical Center, Nashville, TN, and Hiroyasu Inoue, National Cardiovascular Center Research Institute, Osaka, Japan (31). pTOP and pPOF luciferase constructs (for the β-catenin/Tcf-4 transcriptional assay), the wild-type and mutated APC expression vector, β-catenin expression vector, and N-Tcf4 expression vector were kindly given by Bert Vogelstein (32). The mutated K-ras expression vector was kindly given by Channing Der, University of North Carolina at Chapel Hill, Chapel Hill, NC (33). Luciferase constructs containing the full-length COX-2–3′ ARE were kindly given by Dan Dixon, University of Utah, Salt Lake City, UT (34). PclNeo, PclDNA1.1, and PRL-Tk were purchased from Promega (Madison, WI). A COX-2 promoter-luciferase construct with mutant TBE was synthesized by us using a site-specific mutagenesis kit from Stratagene (La Jolla, CA).

Western Blot Analysis. Fifty μg of lysate were denatured by 10% SDS-PAGE (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes (Immobilon-P; Millipore, Burlington, WI). The human colorectal cancer cell lines, HT29-APC and HT29-GAL, were generous gifts from Bert Vogelstein, The Johns Hopkins University Medical Institutions, Baltimore, MD (29). HT29, SW480, and HuH7 were obtained from American Type Culture Collection (Rockville, MD). The HCA7 cell line was kindly given by Susan Kirkland, Imperial College School of Medicine, London, UK (30). For culture of the HT29-APC cells, 0.6 mg/ml of hygromycin (Roche, Indianapolis, IN) was added. To induce full-length APC, HT29-APC cells were incubated with 100 μM of zinc chloride (Sigma, St. Louis, MO).

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Western Blot Analysis. Fifty μg of lysate were denatured by 10% SDS-PAGE (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose filters or polyvinylidene difluoride membranes (Immobilon-P; Millipore, Burlington, MA). The following antibodies were used for immunodetection: monoclonal anti-human COX-2 (Cayman Chemical, Ann Arbor, MI), monoclonal antihuman APC (Oncogene Research, Boston, MA), and monoclonal antihuman actin (Roche). Immunoreactive protein was detected using Western blotting detection reagents (Amersham, Arlington Heights, IL).
PGE2 Assay. Cells were plated in six-well plates at 1 × 10^6 cells/well and grown for 24 h. Cells were incubated with fresh serum-free medium containing 25 μM of arachidonic acid (Cayman Chemical) for the final 1 h. The conditioned medium was removed from the cells and immediately placed at −70°C until PGE2 concentration was measured by ELISA (Cayman Chemical).

Semiquantitative RT-PCR and Northern Blot Analysis. Two μg of total RNA was reverse-transcribed using RETROscript (Ambion, Austin, TX). RT-PCR for COX-2 amplification was performed using Gene-Specific Relative RT-PCR kit and SuperTaq (Ambion) according to the instructions. The PCR conditions were 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s. Exponential amplification had been confirmed up to 35 cycles of the amplification. PCR reaction products were subjected to electrophoresis on 2% agarose gels, stained with ethidium bromide. The amount of amplified product was confirmed by this method to be in linear range with respect to the input RNA for both COX-2 and 18S rRNA primers (Ambion). For Northern blot analysis, 10 μg of total RNA was electrophoresed on 1.2% agarose gel with formaldehyde. RNA was transferred onto Hybond-N membrane (Amersham) overnight. We labeled 50 ng of human cDNA GAPDH and COX-2 probes (Cayman Chemical) with a random priming method using PrimeIt RnT Random Primer Labeling Kit (Stratagene). The membrane was hybridized in QuikHyb Hybridization Solution (Stratagene) at 68°C for 1 h. After washing, the membrane was exposed to X-ray film for 3 days (COX-2) or 3 h (GAPDH), and we quantified the signal intensities using Science Lab 99 Image Gauge version 3.45 (Fuji Photo Film Co., Japan).

EMSA. Recombinant Tcf-4 was expressed in Escherichia coli as a GST fusion protein. EMSA was performed using the following oligonucleotides.

We first used 5'-GCTATGGTACTTGGATCATGATGCTCA-3', which contains a consensus TBE (italicized) of the human COX-2 gene (16).

Secondly, we used 5'-GCTATGGTACTTGGATCATGATGCTCA-3', which has two point mutations (italic) in the TBE. GST-Tcf-4 fusion protein (250 ng) was incubated with 0.2 pmol 32P-end-labeled oligonucleotide in a binding buffer for 30 min. The DNA-protein complex was separated from free oligonucleotide on a 6% nondenaturing polyacrylamide gel. After electrophoresis, the gel was dried, and visualization and quantitation of radioactive bands were performed by a phosphorimager. Binding specificity was checked by incubating in the presence of an excess of nonlabeled oligo (10 and 20 times). For the super-shift assay, 0.5–2 μg of monoclonal anti-Tcf-4 antibody (Ab-3; Calbiochem) was added.

Tcf-4 and COX-2 Transcriptional Activity. Cells were seeded at 1 × 10^6 cells/well the day before transfection. LipofectAMINE (Life Technologies, Inc., Rockville, MD) was used for cotransfection to cell lines with each of the reporter constructs (COX-2 promoter-luciferase constructs, pTOP, and pP0) and 1 ng of pRL-TK renilla luciferase plasmid was used as an internal control. After 3 h of transfection, cells were resuspended in adequate medium containing 10% serum and allowed to recover for 24 h. Firefly and renilla luciferase activities were determined by following a dual-luciferase reporter assay system (Promega). Relative luciferase activities were expressed by dividing firefly luciferase activity with renilla luciferase activity for each sample.

Immunofluorescence. Cellular β-catenin was localized by immunofluorescence, as described elsewhere (35), with an anti-β-catenin monoclonal antibody (Transduction Laboratories, Lexington, KY). In brief, cells were seeded in glass slides and fixed with paraformaldehyde. After being blocked with PBS, supplemented with 5% BSA, slides were incubated with anti-β-catenin monoclonal antibody and subsequently with a donkey antimouse IgG conjugated with Texas Red (The Jackson Laboratory, West Grove, PA). Slides were mounted by Vectashield (Vector Laboratories, Burlingame, CA) with 4',6-diamidino-2-phenylindole and analyzed under a fluorescent microscope.

RESULTS

APC Down-Regulates COX-2 mRNA and Protein. To determine the effect of APC status on COX-2 expression, we first analyzed the HT29-APC cell line, which contains a metastalltoxin promoter-driven, wild-type APC that is activated by zinc (29). HT29-APC induced a full-length APC after incubation with 100 μM zinc (Fig. 1A). We performed Western blot analysis of COX-2 using the lysates both before and after treating the cells with zinc. As shown in Fig. 1B, COX-2 protein was expressed in HT29 cells before induction and subsequently decreased after APC expression. However, the level of COX-2 did not change in the lysates of HT29-APC when it was not treated with zinc (without any induction of wild-type APC expression). We also used HT29-GAL as a control cell line in which β-galactosidase is induced instead of full-length APC. In this cell line, the COX-2 protein levels were not altered after incubating with zinc (data not shown). We measured PGE2 production, which is known to be a major end product of the COX-2-catalyzed reaction. When cells were cultured without zinc, PGE2 levels increased in a time-dependent manner; however, PGE2 was down-regulated 24 h after the induction of wild-type APC expression (Supplemental Fig. 1). These data indicate that following full-length APC induction, COX-2 expression is down-regulated in HT29-APC cells. Based on earlier reports that COX-2 regulation is related to its transcriptional level (36, 37), we hypothesized that APC regulates COX-2 mRNA at the transcriptional level. A previous report demonstrated that expression of full-length APC down-regulated COX-2 protein levels (38). To additionally investigate the mechanism of COX-2 down-regulation, we first performed RT-PCR analysis. Under conditions where the PCR product has not reached a plateau, we compared COX-2 mRNA levels in the samples. COX-2 mRNA was down-regulated after inducing wild-type
There was no significant change in the COX-2 mRNA level in nontreated cells. Then, we did Northern blot analysis of COX-2 (Fig. 1D). Although COX-2 mRNA levels by Northern analysis were reduced in HT29 APC cells incubated without zinc for 24 h, APC induction by zinc more significantly down-regulated COX-2 mRNA levels. COX-2 mRNA was not altered in HT29-GAL after treating with zinc.

**GST-Tcf-4 Fusion Protein Binds to the Tcf-4 Consensus-binding Element in the EMSA.** We analyzed the human COX-2 promoter sequence and identified a possible TBE (Ref. 16; Fig. 2A). This TBE, CTTTGAT, was located at −1079 bp upstream from the transcription start site. Then, we performed EMSA using GST-Tcf-4 fusion protein. We prepared 27 base-paired oligonucleotides; one contained wild-type TBE and the other had two missense mutations. As shown in Fig. 2B, GST-Tcf-4 protein bound to the wild-type TBE oligonucleotide (Fig. 2B, Lane 4), but not to the mutant TBE (Fig. 2B, Lane 3). The addition of nonlabeled wild-type oligonucleotide (10- and 20-fold in excess) dramatically inhibited the binding of TBE and Tcf-4 in a dose-dependent fashion (Fig. 2B, Lanes 5 and 6). However, the mutant nonlabeled oligonucleotide had no effect on the binding (Fig. 2B, Lanes 7 and 8). When incubated with anti-Tcf-4 antibody, the binding complex was super-shifted (Fig. 2B, Lanes 9–11). In addition, after adding a nonlabeled wild-type oligo, both bands disappeared (Fig. 2B, Lane 12). These results clearly show that Tcf-4 can bind specifically to TBE in a COX-2 promoter in vitro.

**CRT Is Down-Regulated by APC Induction in HT29-APC Cells.** Wild-type APC can bind to β-catenin, resulting in the down-regulation of CRT (32). To ascertain if CRT is inhibited after APC induction in our experiments, two sets of reporter constructs for a β-catenin/Tcf-4 reporter gene assay (pTOP and pFOP; Ref. 32) were transfected into HT29-APC. pTOP contains three wild-type TBEs to which β-catenin/Tcf-4 binds, whereas the TBEs in pFOP are mutated and abrogate β-catenin/Tcf-4 binding. As shown in Fig. 3, in the absence of APC, the wild-type TBE luciferase construct (pTOP) showed higher luciferase activity than the mutant TBE luciferase construct (pFOP). The induction of APC significantly decreased the luciferase activity of the wild-type TBE luciferase construct, whereas no reduction was observed in another construct. These results indicate that expression of functional APC causes the down-regulation of β-catenin/Tcf-4-regulated transcriptional activity in HT29-APC cells.

**COX-2 Luciferase Activity Is Down-Regulated by the Expression of APC in HT29-APC Cells.** We hypothesized that COX-2 is one of the downstream target genes of the β-catenin/Tcf-4 pathway, which in turn, is regulated by APC expression. To test this hypothesis, we used several COX-2 promoter-luciferase constructs (31). As shown in Fig. 4A, all of these constructs have three known consensus sequences, NFκB, NF-IL6, and CRE, which are transcriptional regulatory elements of COX-2. The 1.5-kb construct has wild-type TBE upstream of these three binding elements. The 0.4-kb construct does not contain TBE. We made a TBE mutant COX-2 luciferase construct,
in which two bases of TBE were mutated by direct-site mutagenesis method. The 1.5-kb COX-2 luciferase construct with either wild-type or mutant TBE was transfected into the HT29-APC cells, and luciferase activity was measured. Without zinc induction of APC, the luciferase activity of the 1.5-kb COX-2 luciferase construct with wild-type TBE was higher than the construct with mutant TBE. However, with the induction of full-length APC, luciferase activity decreased in the COX-2 luciferase construct containing wild-type TBE. No change in luciferase activity was found with COX-2 luciferase construct containing mutant TBE (Fig. 4B). We also used 0.4-kb luciferase construct, which has no TBE, and the results were similar (data not shown). Our results demonstrate that wild-type APC partially abrogated the transcriptional activity of the COX-2 promoter, and this effect is dependent on the Tcf-4 binding to TBE.

**APC Expression Down-Regulates COX-2 Luciferase Activity in SW480 Cells.** To additionally confirm that APC down-regulates COX-2 expression, we cotransfected either the wild-type or mutant APC expression vector with the COX-2 promoter constructs into the SW480 colon cancer cell line. The mutant APC expression vector produces APC1309 protein, which lacks the β-catenin-binding site, and it was shown that this truncated protein does not target β-catenin for degradation (39). As reported previously (39), wild-type APC expression down-regulated the β-catenin/Tcf-4-regulated transcriptional activity in SW480 cells (data not shown). As shown in Fig. 5, expression of wild-type APC diminished the activity of the 1.5-kb COX-2 promoter-luciferase construct containing wild-type TBE, whereas the activity of the 1.5-kb COX-2 promoter construct containing the mutant TBE was not reduced.

**β-Catenin Up-Regulates COX-2 mRNA Expression in HuH7 Cells.** The data described above indicate that wild-type APC down-regulates COX-2 transcriptional activity by reducing β-catenin/Tcf-4 binding to TBE in the COX-2 promoter. We then conducted experiments to clarify the relationship between β-catenin and COX-2. We chose the HuH7 HCC cell line, which does not show β-catenin nuclear accumulation (40). HuH7 cells were transfected with the mutant β-catenin expression vector, which is not degraded by APC mediation (32, 39). Immunocytochemistry of β-catenin demonstrated that β-catenin was mainly localized in the cytoplasm in mock-transfected HuH7 cells (Supplemental Fig. 2). After transfection of HuH7 with the mutant β-catenin expression vector, β-catenin accumulated in the nucleus (Supplemental Fig. 2). Western blot analysis showed that the total amount of β-catenin (cytoplasmic and nuclear) increased after mutant β-catenin expression vector transfection (data not shown). The mutant β-catenin expression vector caused significant up-regulation of wild-type TBE luciferase activity (Fig. 6A). A deletion mutant of Tcf-4 (NhTcf-4) lacking 30 amino acids at the NH2-terminal end, which contains the β-catenin-binding site, was used as a dominant-negative Tcf-4 expression vector (32), and cotransfection with NhTcf-4 partially inhibited the effect by mutant β-catenin expression (Fig. 6A). In contrast, in the mutant TBE luciferase construct, there was no change mediated by the mutant β-catenin expression vector. We also measured COX-2 promoter-driven luciferase activity. In HuH7 cells transfected with the 1.5-kb COX-2 promoter containing
TBE, the luciferase activity increased when cotransfected with the mutant β-catenin expression vector. Also, the NhTcf-4 vector partially abrogated this effect (Fig. 6B). As expected, using the COX-2 promoter construct containing the mutated TBE, COX-2 luciferase activity increased only slightly and was not down-regulated by the NhTcf-4 vector. When HuH7 cells were transfected with the mutant β-catenin expression vector, the COX-2 mRNA was up-regulated (Fig. 6C). However, COX-2 protein was not detectable in Western blot analysis (Fig. 6D). Then, we used the mutant K-ras expression vector, because Sheng et al. (28) reported that mutant K-ras can stabilize COX-2 mRNA in some cell lines. We first performed luciferase assays using a luciferase construct containing the COX-2–3′-ARE, which has been shown to be involved in COX-2 mRNA stability (41). Mutant K-ras alone or the combination of mutant β-catenin and mutant K-ras caused significantly higher activity of COX-2–3′-ARE luciferase construct, suggesting increased stabilization of COX-2 mRNA in HuH7 cells (Fig. 6E). Then, we again performed RT-PCR and Western blot analyses using mutant K-ras expression vector. Either the mutant K-ras expression increased the COX-2 mRNA or mutant β-catenin significantly up-regulated COX-2 mRNA transcripts (Fig. 6C). However, both mutant β-catenin and mutant K-ras expression were required to increase COX-2 protein levels in the HuH7 cells (Fig. 6D). ELISA of PGE2 also demonstrated that only this combination caused detectable production of PGE2 (Supplemental Fig. 3).

DISCUSSION

We have demonstrated that COX-2 can be regulated by the Wnt-signaling transduction pathway and can cooperate with the ras signal transduction pathway. These results are consistent with the accumulating evidence demonstrating the relationship between COX-2 expression and mutations in the APC or K-ras genes (5, 22–25, 40). Although COX-2 expression is regulated by both the transcriptional and post-transcriptional mechanisms, transcriptional regulation may play a more decisive role in the COX-2 expression in human colon carcinoma cells (36, 37). The COX-2 promoter contains three known
consensus sequences for NFκB, NF-IL6, and CRE (31), which may cooperate in the transcriptional regulation of COX-2. In addition to these three elements, we found that the COX-2 promoter also contains a consensus TBE. Previously, c-myc, cyclin D1, and PPARδ have been identified as downstream targets of the Wnt-signaling pathway (16–19). The promoters of these downstream genes all contain TBE sites. We hypothesized that COX-2 is also a downstream target of the β-catenin/Tcf4-signaling pathway.

A previous report (38) indicated that APC expression decreased the COX-2 protein level by a post-transcriptional mechanism. Our Northern blot and RT-PCR analyses suggest that COX-2 mRNA levels can be diminished by APC induction, and that both β-catenin/Tcf-4-regulated transcriptional activity and COX-2 promoter luciferase activity also were down-regulated. Furthermore, COX-2 luciferase activity was not altered in another COX-2 promoter luciferase construct lacking TBE, suggesting that this down-regulation is TBE-dependent. We also found similar findings using the SW480 cell line transfected with the APC expression vector. Taken together, our results suggest that APC can down-regulate COX-2 mRNA through the β-catenin/Tcf4-signaling pathway.

We also investigated the regulation of COX-2 by the Wnt pathway using the HuH7 liver cell line to extend our results to cells from another organ site (40). Immunocytochemistry showed that β-catenin translocates to the nucleus after transfection of the mutant β-catenin expression vector, and up-regulates both β-catenin/Tcf-4-regulated transcriptional activity and COX-2 promoter activity. Interestingly, the COX-2 luciferase construct with TBE shows higher up-regulation than that without TBE. The cotransfection of a dominant-negative Tcf-4 expression vector diminished this effect, indicating that the up-regulation is, at least in part, Tcf-4-binding-site dependent. These results indicate that nuclear β-catenin accumulation caused the up-regulation of COX-2 mRNA through the β-catenin/Tcf-4-signaling pathway. Although COX-2 mRNA transcripts increase, we detected neither COX-2 protein nor enzymatic activity after expression of mutant β-catenin alone. One possibility is that the COX-2 protein, induced by β-catenin alone, might be below the detectable amount of Western blot analysis. Another explanation is related to the post-transcriptional regulation of COX-2 (41, 42). The 3′-untranslated region of COX-2 mRNA contains multiple copies of AREs that regulate mRNA degradation (41). Ras mutations are found in a wide variety of human malignancies including ~50% of the colorectal cancers (43). Expression of mutant K-ras activates the Raf/mitogen-activated protein/ERK kinase/ERK pathway that results in increased transcription of COX-2 in a rat intestinal epithelial cell line (28). Oncogenic K-ras also activates the phosphatidylinositol 3′-kinase/Akt/PI3K pathway, which cooperates with the mitogen-activated protein/ERK kinase/ERK pathway and results in post-transcriptional stabilization of COX-2 mRNA (28). The luciferase construct containing COX-2 3′-ARE has been used to measure COX-2 mRNA stability (28). We used this strategy and demonstrated that mutant K-ras increased COX-2 mRNA stability in HuH7 cells. Although neither mutant K-ras nor mutant β-catenin expression resulted in detectable COX-2 protein, the combination significantly increased both COX-2 protein and its enzymatic production of PGE2 to biologically active concentrations (44). Taken together, β-catenin can cooperate with K-ras to increase COX-2 mRNA expression and COX-2 protein.

Although APC mutations are positively correlated with increased COX-2 expression (1, 5, 22, 25), the precise mechanism remains unclear. Our present study indicates a mechanistic link between APC and COX-2 through the Wnt-signaling pathway. However, COX-2 transcriptional and translational regulation is complex (36, 45). Additional studies are required to clarify the complexity of COX-2 regulation by β-catenin.

Somatic mutations in APC, and to a lesser extent β-catenin and its nuclear translocation, are observed frequently in colon cancer (46–49). Therefore, the activation of the Wnt signal transduction pathway caused by either APC or β-catenin gene mutation may play a significant role in colon carcinogenesis (50). Compared with colorectal cancer, somatic mutations of APC are rare in HCCs, but APC germ-line mutations are more common in hepatoblastomas (51). Somatic mutations of β-catenin occur in ~20% of HCCs, especially in HCCs associated with HCV infection (52). Axin mutations are also observed in HCCs (40). These studies support the involvement of the Wnt pathway in human liver carcinogenesis. Although the role and mechanism of increased COX-2 in HCCs is still uncertain (53, 54), our data provide molecular evidence that the Wnt pathway is a mechanism for COX-2 regulation during hepatocarcinogenesis. The cooperation between the Wnt and ras pathways in COX-2 regulation may also be involved in the molecular pathogenesis of HCCs. For example, increased expression of insulin-like growth factor in HCCs (55) may stimulate both the Wnt and the ras pathways (56).

In conclusion, COX-2 can be down-regulated by APC induction and up-regulated by the nuclear accumulation of β-catenin. Both of these effects might be related to transcriptional regulation through the Wnt-signaling pathway. We also provide novel evidence indicating the cooperation of the Wnt- and ras-signaling pathways in the regulation of COX-2 expression.

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