Hepatocellular carcinoma accounts for ~6% of all human cancers (1, 2). The increasing incidence and poor prognosis of hepatocellular carcinoma (3, 4) emphasize a need to explore an effective chemoprevention for this disease. Overexpression of cyclooxygenase-2 (COX-2) is associated with carcinogenesis in colorectal, prostate, and breast cancers, and in hepatocellular cancers (1, 2). The frequency of aberrant COX-2 expression plays an important role in hepatic inflammation and malignant transformation of hepatocytes.

Both in vitro and in vivo studies have shown that selective COX-2 inhibitors can effectively suppress proliferation of colon and prostate cancer and other malignancies, including hepatocellular carcinoma (10–15). A clinical trial further showed a chemopreventive effect of celecoxib on colon cancer (16). This has promoted extensive studies on testing COX-2 inhibitors for chemoprevention of various malignancies (6, 17–19).

We and other investigators have shown that COX-2 inhibitors, including NS-398, celecoxib, and meloxicam, effectively inhibit hepatocellular carcinoma cell growth both in vitro and in vivo (10–13, 20, 21). Although these studies have shown that COX-2 inhibitors suppress hepatocellular carcinoma cell growth by affecting cell cycle progression and apoptosis, the precise mechanisms remain unknown on how COX-2 inhibitors precisely affect these pathways.

Studies from colon cancer and other malignancies have indicated that COX-2 inhibitor–induced growth inhibition of cancer cells seems to be mediated by both COX-2-dependent and COX-2-independent pathways (15, 17, 22, 23). However, it remains unclear whether COX-2-independent pathway [e.g., peroxisome proliferator-activated receptor γ (PPARγ)] plays important roles in COX-2 inhibitor–induced growth inhibition of hepatocellular carcinoma cells.
In the present study, two human hepatocellular carcinoma cell lines, HuH7 (high COX-2 expression) and PLC/PRF/5 (PLC; low COX-2 expression) cells (10), were used to examine the effect and molecular mechanisms of celecoxib on the growth of both cells. Our results indicate that celecoxib suppresses growth of the hepatocellular carcinoma cells in vitro by inhibition of retinoblastoma phosphorylation and formation of DP1/E2F1 complex and by activation of caspase-3 and caspase-9 that are both COX-2 dependent and independent. Furthermore, we show that celecoxib partially suppresses growth of HuH7 xenografts in nude mice.

Materials and Methods

Reagents. DMEM, fetal bovine serum, trypsin-EDTA, and penicillinstreptomycin-fungizone were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD). Prostaglandin E2 (PGE2), DMSO, and anti-active caspase-3 were from Sigma Chemical Co. (St. Louis, MO). The 5-bromo-2-deoxyuridine (BrdUrd) and the cell death detection kits were from Roche Applied Science (Indianapolis, IN). Celecoxib was kindly provided by Pharmacia & Upjohn Co., a Division of Pfizer, Inc. (Kalamazoo, MI). The antihuman COX-2 was from Cayman Chemical Company (Ann Arbor, MI). The antibodies against human cyclin D1 (CD1), DP1, phosphorylated retinoblastoma, E2F-1, cyclin-dependent kinase-4 (CDK4), p21waf1/cip1 and p27kip1, PPARg, active caspase-9, Bcl-2, and β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence Western blotting detection reagents and PGE2 enzyme immunoassay system were from Amersham Biosciences Corp. (Piscataway, NJ).

Cell culture. Both HuH7 and PLC cells were cultured in DMEM with 10% fetal bovine serum and penicillin-streptomycin-fungizone (10). The experiments were done when cells reached ~80% confluence and cultured in fetal bovine serum–free media (i.e., serum starved) for 24 hours.

Cell proliferation assay. Cell proliferation was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Titer 96 AQ ueous One Solution Reagent, Promega Corporation, Madison, WI) and ELX800 Universal Microplate Reader (Bio-Tek Instruments, Inc, Winooski, VT) as previously reported (10). To examine the effect and mechanisms of celecoxib-induced growth inhibition of human hepatocellular carcinoma cells, the IC50 of celecoxib was determined—70 μmol/L for HuH7 cells and 61 μmol/L for PLC cells. To determine the pattern of time-dependent growth inhibition of HuH7 and PLC cells, the respective IC50 dose of celecoxib was added and the culture was maintained from 24 to 120 hours with daily MTT assay (10).

BrdUrd uptake was measured as previously reported (10). Briefly, 10 μL of BrdUrd labeling solution was added to each well in triplicate and were cultured for 2 hours. The extinction at 405 nm and reference wavelength at 490 nm were then measured and ratio of the absorbance was expressed as a percentage. The mean values were used for data analysis.

![Western blot of COX-2 expression in PLC and HuH7 cells. The standard COX-2 was used as positive control and β-actin was used as internal control. The Western blot shows a very low COX-2 expression in PLC but a high COX-2 expression in HuH7 cells.](https://static.aacrjournals.org/cancerres/000/0000/0000000000.png)

**Fig. 1.** Western blot of COX-2 expression in PLC and HuH7 cells. The standard COX-2 was used as positive control and β-actin was used as internal control. The Western blot shows a very low COX-2 expression in PLC but a high COX-2 expression in HuH7 cells.

**Fig. 2.** *In vitro* effect of celecoxib on PLC and HuH7 cell growth. The cell viability, proliferating cell nuclear antigen expression, and BrdUrd uptake were assessed as described in Materials and Methods. A, comparable dose-dependent growth inhibition of both cells by celecoxib regardless of COX-2 expression. B, celecoxib inhibits proliferating cell nuclear antigen expression in both cells. Columns, mean; bars, SD. Western blot of β-actin was used as internal control (data not shown). C, celecoxib induces time-dependent growth inhibition in PLC and HuH7 cells. D, celecoxib induces inhibition of BrdUrd uptake in PLC and HuH7 cells. #, P < 0.05; *, P < 0.01, compared with vehicle control.
Total prostaglandin E$_2$ production in hepatocellular carcinoma cells and the effect of exogenous prostaglandin E$_2$ on celecoxib-induced growth inhibition of hepatocellular carcinoma cells. As previously reported (10), total PGE$_2$ production was determined using a PGE$_2$ growth inhibition of hepatocellular carcinoma cells. As previously used for data analysis. was then determined as described above and the mean values were used for data analysis.

**Analysis of apoptosis.** After treatment with respective IC$_{50}$ dose of celecoxib for 48 hours, apoptosis was determined with two different methods. First, an enzyme immunoassay kit for cell death detection was used as previously reported (10). Second, the activated caspase-3 and caspase-9 and Bcl-2 were measured by Western blot assay as described below.

**Immunoprecipitation and immunoblot analysis.** After treatment with IC$_{50}$ dose of celecoxib, the cell pellets were lysed with lysis buffer on ice for 30 minutes. The lysates were centrifuged and the supernatants were used to detect proliferating cell nuclear antigen, p21$^{WAF1/CIP1}$, p27$^{kip1}$, DP1, E2F1, CD1, CDK4, phosphorylated retinoblastoma, activated caspase-3 and caspase-9, Bcl-2, and PPAR$\gamma$. For immunoprecipitation assays, 200 $\mu$g of cellular protein were incubated with 10 $\mu$L of appropriate rabbit anti-human antibodies at 4°C overnight, followed by addition of 25 $\mu$L of anti-rabbit IgG-conjugated agarose beads for 2 hours at 4°C. The beads were pelleted by centrifugation and washed thrice with the lysis buffer. The samples were subjected to SDS-PAGE and Western blot assay using the primary polyclonal antibodies (in 1:1,000 dilution) and enhanced chemiluminescence Western blotting detection reagents. All the Western blot experiments were repeated thrice. Western blot for $\beta$-actin was used as internal control. To quantify the results, the relative amount of each protein was determined by digitally scanning its hybridizing bands using Scion Image software (Scion Corporation, Frederick, MD). The mean values were used for data analysis.

**In vivo effect of celecoxib on growth of human hepatocellular carcinoma xenografts.** To assess the in vivo suppressive effect of celecoxib on growth of HuH7 cells, a nude mice model bearing hepatocellular carcinoma xenografts was used (24). The animal experiments were approved by our Institutional Animal Care and Use Committee. Four-week-old male NCRNU-M nude mice were from Taconic Farms, Inc. (Hudson, NY) and s.c. inoculated with 5$\times$10$^6$ or 0.25 mL of HuH7 cells in the right flank. The mice received standard rodent chow and water ad libitum and were randomized into four groups with five mice in each group. The control group received daily gavage of a vehicle solution containing 0.5% methylcellulose and 0.025% Tween 20 (25), and the three test groups received gavage of celecoxib at 12.5, 25, or 50 mg/kg/d, respectively, started at the second day of HuH7 cell inoculation. Celecoxib dose was adjusted weekly based on changes in body weight. Tumor volumes were recorded weekly (18). By the end of 5-week observation, the hepatocellular carcinoma xenografts were weighted after euthanasia. A 300 mg of tumor tissue from each xenograft was used to determine in vivo effects of celecoxib treatment on PGE$_2$ content and PPAR$\gamma$ expression.

**Statistical analysis.** The descriptive statistics was provided with mean $\pm$ SD. A repeated-measure ANOVA test was used to assess dose-dependent and time-dependent effects of celecoxib on PLC and HuH7 cells. The Student’s $t$ test or ANOVA test was used to compare frequencies or means, respectively. A $P < 0.05$ was considered statistically significant.

**Results**

In vitro effects of celecoxib on proliferation of human hepatoma cells. As we previously reported, HuH7 cells express a high level of COX-2 (10). Using immunohistochemistry and Western blot, we and other groups could not detect COX-2 expression in PLC cells previously (10, 11). However, using immunoprecipitation, we confirmed a very low level expression of COX-2 in PLC cells. However, using immunoprecipitation, we confirmed a very low level expression of COX-2 in PLC cells. As we previously reported, HuH7 cells express a high level of COX-2 (10). Using immunohistochemistry and Western blot, we and other groups could not detect COX-2 expression in PLC cells previously (10, 11). However, using immunoprecipitation, we confirmed a very low level expression of COX-2 in PLC cells. In the present study (Fig. 1), therefore, PLC cells have a very low COX-2 expression.

Treatment with various doses of celecoxib for 48 hours significantly reduced proliferation of both HuH7 and PLC cells as determined by MTT that was associated with reduced COX-2.
Fig. 4. Effects of celecoxib on PPARγ and modulators of cell cycle in PLC and HuH7 cells. The cells were treated as described in Materials and Methods. Columns, mean of scanning results from three experiments; bars, SD. A, Western blot of β-actin was used as internal control. Celecoxib increases PPARγ expression (B), inhibits formation of DP1/E2F1 complex (C), retinoblastoma phosphorylation (D), and formation of CD1/CDK4 complex (E), and increases p21^waf1/cip1 expression (F) in PLC and HuH7 cells. #, P < 0.05; *, P < 0.01, compared with vehicle control.
Celecoxib promotes apoptosis in PLC and HuH7 cells. Celecoxib significantly promotes apoptosis in both cells. #, P < 0.05; *, P < 0.01, compared with controls.

expression (data not shown). Although the degree of COX-2 expression was different, celecoxib-reduced cell proliferation was dose-dependent in these two cells (Fig. 2A). Western blot analysis revealed that proliferating cell nuclear antigen expression was significantly decreased in both cells after treatment with IC_{50} dose of celecoxib (P < 0.01; Fig. 2B) but the reduced proportion was more in HuH7 (75.8%) than in PLC cells (45.0%, P = 0.01). The IC_{50} dose of celecoxib also resulted in significant time-dependent inhibition of cell proliferation in both cells (Fig. 2C) that was associated with a comparable pattern of decreased BrdUrd uptake (Fig. 2D). Thus, using three different methods, we showed that celecoxib suppressed proliferation of both HuH7 and PLC cells regardless of the degree of COX-2 expression.

Association of prostaglandin E_2 with celecoxib-induced inhibition of proliferation of HuH7 and PLC cells. First, we examined the effect of celecoxib on PGE_2 production. Consistent with the level of COX-2 expression, the basal PGE_2 level was lower in PLC cells than in HuH7 cells (Fig. 3A). After treatment with IC_{50} dose of celecoxib, cell proliferation was suppressed in association with a significantly decreased production of PGE_2 in both cells (Fig. 3A). This indicates that celecoxib suppresses hepatocellular carcinoma cell proliferation by decreased production of PGE_2, a typical COX-2-dependent pathway. Second, we tested the effect of exogenous PGE_2 on celecoxib-reduced hepatocellular carcinoma cell proliferation. As shown in Fig. 3B and C, exposure of HuH7 and PLC cells to both IC_{50} dose of celecoxib and PGE_2 (ranging from 1 to 4 μg/mL) resulted in a partial reversion of celecoxib-mediated inhibition of hepatocellular carcinoma cell proliferation regardless of the degree of COX-2 expression. Although the effect of PGE_2 was dose-dependent, it only partially counteracted the effect of celecoxib. This further indicates that a COX-2-independent pathway was also involved in celecoxib-mediated inhibition of hepatocellular carcinoma cell proliferation.

Effect of celecoxib on peroxisome proliferator-activated receptor γ expression in HuH7 and PLC cells. To further examine if celecoxib-reduced hepatocellular carcinoma cell proliferation involves a COX-2-independent mechanism, we assessed the effect of celecoxib on PPARγ expression in HuH7 and PLC cells. We found that celecoxib significantly increased PPARγ expression in both HuH7 and PLC cells (Fig. 4B).

Molecular effects of celecoxib on cell cycle progression of HuH7 and PLC cells. To further study the molecular effects of celecoxib on cell cycle progression of HuH7 and PLC cells, we assessed how celecoxib affects the expression of a series of cell cycle–related modulators. β-actin was used as an internal control (Fig. 4A). Because DP1/E2F1 plays a critical role in G1-S transition of the cell cycle, we assessed the effect of celecoxib on the formation of this complex. We found that celecoxib significantly inhibited the formation of DP1/E2F1 complex (Fig. 4C). In addition, celecoxib induced a significant inhibition of retinoblastoma phosphorylation in both cells (Fig. 4D). A significantly higher suppression of retinoblastoma phosphorylation was seen in HuH7 cells (93.8%) than in PLC cells (83.1%; P = 0.01). We also confirmed that celecoxib significantly inhibited the formation of CD1/CDK4 complex (Fig. 4E).

Both p21^{waf1/cip1} and p27^{kip1} are two important CDK inhibitors involved in modulating cell cycle in cancer cell growth. As shown in Fig. 4F, celecoxib significantly increased p21^{waf1/cip1} in both cells regardless of the degree of COX-2 expression. However, celecoxib did not alter p27^{kip1} expression (data not shown).

In vitro effects of celecoxib on apoptosis of HuH7 and PLC cells. Two different methods, including cell death assay and activation of caspase-3 and caspase-9, and Bcl-2 expression, were used to assess the effects of celecoxib on apoptosis of HuH7 and PLC cells. Celecoxib resulted in a significant increase of apoptosis in both cells as determined by a quantitative enzyme immunoassay of cytoplasmic histone-associated DNA fragment (Fig. 5) but the increased proportion was more in PLC (4.6-fold) than in HuH7 cells (2.2-fold; P = 0.01).

To further elucidate the mechanisms of celecoxib-induced apoptosis in HuH7 and PLC cells, execution events of downstream apoptosis were analyzed. Although celecoxib significantly increased activated caspase-3 and caspase-9 (Fig. 6B and C), it significantly down-regulated expression of Bcl-2 protein in both cells (Fig. 6D and E).

In vivo effects of celecoxib on growth of HuH7 xenografts in nude mice. The effect of celecoxib on body weight, frequency, and tumor weight of hepatocellular carcinoma xenografts in nude mice were summarized in Table 1. Compared with the control group, celecoxib at a dose range from 12.5 to 50 mg/kg/d did not alter the body weight of the mice but reduced the frequency and mean weight of HuH7 xenografts.

Because celecoxib at 50 mg/kg/d was associated with most significant decrease in tumor weight, the xenograft tissues from this group and the control group were used to determine the in vivo mechanisms of celecoxib-mediated growth inhibition of HuH7 xenografts. First, treatment with celecoxib was associated with a significantly reduced PGE_2 production in hepatocellular carcinoma xenograft tissue (Fig. 7A), indicating an in vivo COX-2-dependent pathway. Consistent with the in vitro results, celecoxib increased PPARγ expression in the xenograft tissue, indicating an in vivo mechanism of the COX-2-independent pathway (Fig. 7B).
Discussion

The carcinogenic role of COX-2 is indicated by a high frequency of COX-2 overexpression in various human malignancies (5–7), including hepatocellular carcinoma (8–10). Therefore, COX-2 inhibitors may represent a novel chemoprevention for human malignancies. We reported that NS-398, a preclinical selective COX-2 inhibitor, suppresses proliferation of high COX-2-expressing HuH7 cells but it does not inhibit proliferation of low COX-2-expressing PLC cells (10). Kern et al. (12) reported that celecoxib, a clinically available COX-2 inhibitor, suppresses proliferation of HuH7 cells. Using three different methods, including MTT, proliferating cell nuclear antigen expression, and BrdUrd uptake, we showed that celecoxib resulted in comparable inhibition of proliferation in both cells. Hence, our results showed that celecoxib mediates inhibition of hepatocellular carcinoma cell proliferation regardless of the degree of COX-2 expression.

Several studies have indicated that celecoxib may exert its anticancer effect through both COX-2-dependent and COX-2-independent pathways (15, 17, 22, 23). However, it remains unknown whether the COX-2-independent pathway is involved in celecoxib-reduced hepatocellular carcinoma cell proliferation. To address this issue, the present study generated three lines of evidence. First, although low COX-2 expressing PLC cells produce a lower level of PGE2 than HuH7 cells, celecoxib resulted in comparable inhibition of proliferation in both cells. Second, addition of exogenous PGE2 at the dose as high as 1 to 4 μg/mL resulted in comparable but only partial counteracting effect on celecoxib-mediated suppression of proliferation in both cells. Third, celecoxib induced PPARγ expression in both cells regardless of the degree of COX-2 expression. Thus, our data showed that celecoxib mediates suppression of proliferation of human hepatocellular carcinoma cells through both COX-2-dependent and COX-2-independent pathways. Because not all patients with hepatocellular carcinoma present with COX-2 overexpression (8, 11), our findings provide rationale to further test celecoxib as a hepatocellular carcinoma chemopreventive agent in patients with increased risk for hepatocellular carcinoma regardless of the degree of COX-2 expression.

Fig. 6. Celecoxib induces activation of caspase-3 and caspase-9, and reduces Bcl-2 expression in PLC and HuH7 cells. A, Western blot of β-actin was used as internal control. Celecoxib increases activation of caspase-3 (B) and caspase-9 (C) but reduces Bcl-2 expression (D) in PLC and HuH7 cells. ##, P < 0.05; *, P < 0.01, compared with vehicle control.
G1-S progression serves as one of the most important checkpoints of the cell cycle. Imbalanced G1-S progression results in uncontrolled proliferation, malignant transformation, and carcinogenesis (26, 27). On the other hand, effective inhibition of G1-S progression has been associated with cell cycle arrest and suppression of tumor growth (28). G1-S checkpoint is mainly controlled by the complexes of CD1 with CDKs (i.e., CDK2, CDK4, and CDK6), retinoblastoma phosphorylation, and CDK inhibitors, including p21waf1/cip1 and p27kip1 (28). Phosphorylation of retinoblastoma induced by CD1/CDK complex results in formation of DP1/E2F1 complex that binds to cellular DNA and transactivates respective target genes and promotes cell cycle progression (29). In a hepatocellular carcinoma transgenic mouse model, overexpression of E2F1 has been associated with hepatocarcinogenesis (30).

Various concentrations of celecoxib have been used to test its effect on cell cycle progression of the malignant cells. These may partially explain the inconsistent results reported previously (15–18). In the present study, celecoxib at IC50 dose was used as the standard dose to study how this drug affects HuH7 and PLC cell cycle progression. We found that celecoxib significantly inhibited formation of DP1/E2F1 complex in both cells. Therefore, our results showed that the overall molecular effect of celecoxib-mediated suppression of hepatocellular carcinoma cell proliferation is to suppress G1-S progression by decreasing the formation of DP1/E2F1 complex regardless of the degree of COX-2 expression.

To further disclose molecular mechanisms on how celecoxib affects formation of DP1/E2F1 complex, we examined retinoblastoma phosphorylation, the immediate upstream pathway of DP1/E2F1 complex in G1-S checkpoint. Increased retinoblastoma phosphorylation is considered as the key step in G1-S checkpoint (31). We showed that through COX-2-independent pathway, celecoxib resulted in significant inhibition of retinoblastoma phosphorylation in both cells. Taken together, our results showed that celecoxib suppresses G1-S progression in both cells by inhibiting retinoblastoma phosphorylation and formation of DP1/E2F1 complex by COX-2-independent pathway.

CD1/CDK4 complex represents one of the positive modulators in G1-S progression of the cell cycle. We found that celecoxib significantly inhibited the formation of CD1/CDK4 complex in both cells through COX-2-independent pathway. Both p21waf1/cip1 and p27kip1 serve as negative modulators in G1-S progression of the cell cycle (28, 32, 33). In the present study, we also confirmed that through COX-2-independent pathway, celecoxib significantly increased p21waf1/cip1 expression. Although these results showed the potential roles of altered formation of CD1/CDK4 and p21waf1/cip1 expression in celecoxib-mediated suppression of hepatocellular carcinoma proliferation, additional studies will be needed on how these alterations fit the complicated signal interactions of cell cycle regulation.

Studies have shown that celecoxib induces cancer cell apoptosis (18, 21). We and other research groups have reported that NS-398 and celecoxib promote apoptosis of COX-2-expressing hepatocellular carcinoma cells (10–12). However, it remains to be determined whether celecoxib-mediated apoptosis of hepatocellular carcinoma cell is COX-2 dependent or not, and how celecoxib affects apoptosis in these cells. In the present study, we showed that celecoxib promotes apoptosis in both cells regardless of the degree of COX-2 expression. The promoting effect was higher in PLC cells than in HuH7 cells, suggesting that a higher COX-2 expression in HuH7 cells might...

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*P < 0.05 compared with control group.

Fig. 7. In vivo effects of celecoxib on PGE2 production and PPARγ expression in HuH7 xenografts. PGE2 and PPARγ contents were determined as described in Materials and Methods. Celecoxib at 50 mg/kg/d reduces PGE2 content (A) but increases PPARγ expression (B) in HuH7 xenografts. #, P < 0.05, compared with vehicle control.
counteract celecoxib-induced apoptosis. It was reported that celecoxib induces apoptosis of hepatocellular carcinoma and cholangiocarcinoma cells by modulating the activation of caspase-3 and caspase-9 (12, 18). In the present study, we found that celecoxib resulted in increased levels of activated caspase-3 and caspase-9 but decreased Bcl-2 expression in both cells. Thus, our results confirmed that celecoxib promotes apoptosis of hepatocellular carcinoma cells by modulating activation of caspase-3 and caspase-9 that seems to be not dependent on degree of COX-2 expression. Kern et al. (12) reported that celecoxib-induced apoptosis in hepatocellular carcinoma cells was independent of Bcl-2. However, we found that celecoxib-induced apoptosis of hepatocellular carcinoma cells is also mediated through down-regulation of Bcl-2 expression. Additional study will be needed to address this disparity.

PPARγ is a member of the nuclear hormone receptors. Recent data showed that PPARγ activated by its ligands inhibits cell growth and induces apoptosis in human liver cancer cells (34, 35). PPARγ is a target of COX-2-independent pathway mediated by nonsteroidal anti-inflammatory drugs (23, 36). In the present study, we found that celecoxib increases in vitro and in vivo expression of PPARγ in PLC and HuH7 cells regardless of the degree of COX-2 expression. This provided additional evidence that a COX-2-independent pathway is also involved in celecoxib-mediated growth inhibition of hepatocellular carcinoma cells. A reciprocally negative regulation between PPARγ and COX-2 expression has been reported (37, 38). It is possible that increased PPARγ by celecoxib may further inhibit COX-2 expression and/or activity. Our findings open new insights on the association of celecoxib and hepatocarcinogenesis with signal transduction of the PPARγ pathway.

Recently, Kern et al. (20) reported that meloxicam, another selective COX-2 inhibitor, inhibits growth of hepatocellular carcinoma xenografts in nude mice. Using nude mice bearing HuH7 xenografts, we showed a dose-dependent inhibition of hepatocellular carcinoma xenografts by celecoxib as assessed by frequency and load of hepatocellular carcinoma xenografts. We used daily gavage to deliver accurate dose of celecoxib and showed that celecoxib at 50 mg/kg/d resulted in an optimal reduction of hepatocellular carcinoma xenografts without significant alteration of the body weight in nude mice. It was recently reported that celecoxib at a high dose could be associated with increased cardiovascular adverse event (39). Celecoxib at the dose of 50 mg/kg/d in mice is considered equivalent to a dose of 200 mg/d for patients (19). Based on these data, we assume that hepatocellular carcinoma chemoprevention might be achievable in human beings using a low dose of celecoxib (i.e., 200 mg/d).

Consistent with our in vitro results, celecoxib-reduced growth of HuH7 hepatocellular carcinoma xenografts is associated with decreased PGE2 production and increased PPARγ expression. This provides in vivo evidence that celecoxib suppresses hepatocellular carcinoma growth through both COX-2-dependent and COX-2-independent pathways.

In summary, our results showed that celecoxib suppresses human hepatocellular carcinoma cell proliferation effectively through both COX-2-dependent and COX-2-independent pathways. This is mediated by (a) decreased retinoblastoma phosphorylation and formation of DP1/E2F1 complex, the key checkpoint of G1-S progression; (b) increased activation of caspase-3 and caspase-9 but decreased Bcl-2 expression, the key steps for apoptosis; and (c) increased expression of PPARγ, a novel COX-2-independent pathway associated with cell proliferation. It was recently reported that celecoxib at a high dose could be associated with increased cardiovascular adverse event (39). However, it remains to be determined whether a low dose of celecoxib alone or combined with other agents serves as a potential approach for cancer chemoprevention (40). Our study showed that celecoxib, at a low dose equivalent to current recommendation for arthritis, causes potent in vivo growth inhibition of hepatocellular carcinoma xenografts in nude mice that is associated with reduced PGE2 production and increased PPARγ expression.

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In vitro and In vivo Effects and Mechanisms of Celecoxib-Induced Growth Inhibition of Human Hepatocellular Carcinoma Cells

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