

Direct Evidence for a Role of Cyclooxygenase 2-derived Prostaglandin E₂ in Human Head and Neck Xenograft Tumors

Ben S. Zweifel, Thomas W. Davis, Richard L. Ornberg, and Jaime L. Masferrer

Pharmacology/Oncology, Pharmacia Corporation, St. Louis, Missouri 63198

ABSTRACT

Both nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX) 2-selective inhibitors such as celecoxib are being reported as having potent anticancer activity in laboratory models. Several reports have suggested that the mechanism of action of these agents in reducing tumor volume/burden is unrelated to their inhibition of prostaglandin synthesis. Many *in vitro* reports use supraphysiological concentrations of these drugs to demonstrate COX-independent activities on apoptosis or proliferation. *In vivo*, most murine tumor models express COX-2 only in the vasculature and stroma, unlike human tumors that also express COX-2 in the tumor cells. In general, these models have the limitation of having no measurable, COX-2-derived, prostaglandins, the inhibition of which correlates with antitumor efficacy. We report here that 1483 human head and neck xenograft tumors express COX-2 similar to the pattern observed in human solid tumors and that COX-2 activity produces high levels of prostaglandin E₂ (PGE₂). Inhibition of COX-2 by celecoxib resulted in loss of intratumor PGE₂ levels and reduced tumor growth in a dose-dependent manner. In contrast, a selective COX-1 inhibitor, SC-560, did not measurably reduce tumor prostaglandin levels or tumor growth despite the presence of COX-1 in the host and tumor cells. Celecoxib-treated tumors showed reduced proliferation and increased apoptosis of both tumor and stromal cells compared with vehicle controls. Specific inhibition of PGE₂ activity by a neutralizing antibody mimicked the reduced tumor growth observed after celecoxib treatment, suggesting growth is PGE₂ mediated. These data indicate that a major antitumor mechanism of action of celecoxib is inhibition of COX-2-derived prostaglandins, particularly PGE₂, and suggest celecoxib as a novel therapeutic agent for human head and neck cancer.

INTRODUCTION

Inflammatory mediators such as cytokines, eicosanoids, and growth factors are thought to play a critical role in the maintenance, survival, and growth of tumor cells (1, 2). One major class of mediators, prostaglandins, is produced from arachidonic acid by an initial reaction with either of two enzymes, COX²-1 or COX-2, followed by prostaglandin synthase. In particular, PGE₂, COX-2, and microsomal PGE synthase have been linked with cancer (3). COX-1 is constitutively expressed in most tissues, whereas COX-2 is induced in association with pathological inflammatory sites, including human cancers (1, 4). Both COX isozymes can be inhibited by traditional NSAIDs such as aspirin and ibuprofen. Studies have shown that regular intake of aspirin or other NSAIDs provides a 40–50% reduction in relative risk of death by colon cancer (5, 6). Other epidemiological studies demonstrate up to a 90% decreased risk of developing esophageal cancer by chronic use of aspirin (7, 8), suggesting that inhibition of

COX in humans can have a chemopreventive effect (5). The importance of the COX-2 isoform in tumorigenesis was first demonstrated by the observation that, in rodent models of familial adenomatous polyposis, a genetic disease leading to GI cancer, loss of COX-2 activity by either genetic deletion or selective enzymatic inhibition suppressed intestinal polyp formation (9, 10). Celecoxib, a COX-2 inhibitor, has been approved in humans for adjunctive therapy in this population. Preclinical studies using selective COX-2 inhibitors have demonstrated chemopreventive activity in animal models of colon, bladder and breast cancer (11–13). Additional evidence for the importance of COX-2 in tumorigenesis was reported by Hla *et al.* (14), who showed that selective COX-2 overexpression in the mammary gland of transgenic mice led to tumorigenesis. Taken together, these data provide strong evidence for the importance of COX-2 enzyme activity in oncogenesis and as a target of NSAID antitumor action.

COX-2 has been shown to be expressed in neoplastic epithelial cells in a wide variety of human tumor types (1, 15, 16). It has also been shown that different types of epithelial cancers produce high levels of PGE₂ (17, 18). Also, PGE₂ and its metabolite (13,14-dihydro-15-keto-PGE₂) was reported to be elevated in the urine and plasma of cancer patients (19, 20). Analysis of human tumor tissue has demonstrated that PGE₂ levels can be elevated substantially over normal surrounding tissue (21). Additionally, human HNSCC has been reported to have elevated levels of COX-2 mRNA, protein, and PGE₂ compared with surrounding tissues (22–25). Zimmerman *et al.* (25) demonstrated that a COX-2-expressing HNSCC cell line produced 600 times more PGE₂ than a non-COX-2 expressing line and that inhibition of PGE₂ by two COX inhibitors resulted in reduced proliferation and increased apoptosis *in vitro* of the COX-2-expressing line. Similarly, Sumatani *et al.* demonstrated that COX-2 inhibition limited growth of an oral cancer cell line expressing high levels of COX-2. (26). Growth of another cell line, which expressed only low levels of COX-2, was not affected by treatment. Although, in this study, the levels of inhibitor reported were higher than would be expected to be necessary for COX-2 inhibition, similar results were obtained with COX-2 antisense oligonucleotides and growth was rescued by addition of exogenous PGE₂.

In addition to neoplastic epithelium, COX-2 is highly expressed in the tumor microvasculature in many human tumors (1, 15, 16). This suggests that COX-2-derived PGE₂ may also support tumor growth through angiogenic endothelial cell growth and blood vessel formation (27, 28). This hypothesis is supported by the observation that celecoxib inhibits fibroblast growth factor-mediated angiogenesis in a rat corneal micropocket assay (29). We have previously reported that the COX-2 inhibitor, celecoxib, inhibits tumor growth in a number of murine tumor models. However, in these models, COX-2 could only be detected in the neovasculature and not the tumor cells. Because COX-2-derived PGE₂ synthesis is not measurable in these models, it has been speculated that COX-2 inhibitors may act independently of inhibition of prostaglandin synthesis. This controversy has been fostered by reports of COX-independent activities of traditional NSAIDs, particularly *in vitro* at high micromolar concentrations (30–32). We hypothesize that the primary mechanism of action of celecoxib within these models is believed to be antiangiogenic (16, 29, 33) elegantly supported by Williams *et al.* (34), who showed that host COX-2 is

Received 5/16/02; accepted 9/19/02.

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

¹ To whom requests for reprints should be addressed, at Fellow Scientist, Pharmacology/Oncology, Pharmacia Corporation, 700 North Chesterfield Village Parkway, AA5A, St. Louis, MO 63198. Phone: (636) 737-6910, Fax: (636) 737-5574; E-mail: jaime.l.masferrer@pharmacia.com.

² The abbreviations used are: COX, cyclooxygenase; PGE₂, prostaglandin E₂; NSAID, nonsteroidal anti-inflammatory drug; GI, gastrointestinal; TxB₂, thromboxane B₂; TxA₂, thromboxane A₂; HNSCC, head and neck squamous cell carcinoma; EIA, enzyme immunoassay; BrdUrd, bromodeoxyuridine; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; TBS, Tris-buffered saline; 5-FU, 5-fluorouracil.

important for tumor neovascularization and subsequent tumor growth. However, because most human cancers express COX-2 in both the neoplastic and the stromal cells, the current models do not fully reflect the complexity of human cancer (16). In this paper, we demonstrate that COX-2-derived PGE₂ drives tumor growth in a xenograft model of HNSCC with COX-2 expression that occurs in both stromal (inflammatory and neovascular) and neoplastic cells and has measurable COX-2-derived prostaglandin levels. Inhibition of PGE₂ synthesis with celecoxib or neutralizing PGE₂ activity with an antibody to PGE₂ inhibited tumor growth in this model, suggesting that tumor growth is PGE₂ dependent. Furthermore, inhibition of PGE₂ production by celecoxib is correlated with increased apoptosis and decreased proliferation in both tumor and stromal cells.

MATERIALS AND METHODS

1483 Cell Culture. Human HNSCC 1483 cells were derived from the retromolar trigone from an untreated patient with nodal metastasis (35). Cells were grown in standard tissue culture conditions in DMEM/F-12 media (Life Technologies, Inc.) supplemented with 1% HEPES buffer, 2 mM L-glutamine, pyridoxine hydrochloride, 10% FBS, and 0.05 mg/ml gentamicin (Life Technologies, Inc.). Cells were fed every other day, passaged at 80–90% confluence and not maintained beyond passage 10.

In Vivo Tumor Studies. HNSCC 1483 cells (1×10^6) suspended in 30% Matrigel (BD Biosciences) in HBSS (Life Technologies, Inc.) were implanted into the right hind paw of athymic male nude mice ($n = 8/\text{group}$). Celecoxib (160 and 1600 ppm equivalent to 25 and 250 mg/kg/day p.o., respectively) and SC-560 (20 ppm equivalent to 3 mg/kg/day p.o.) therapy was initiated in the diet when tumors reached a mean volume of 0.1 ml and maintained for the duration of the experiment. A mouse anti-PGE₂ monoclonal antibody, 2B5, or isotype-matched IgG control antibody, MOPC31, was administered at 10 mg/kg 3 times/week i.p. when tumor reached a mean volume of 0.1 ml and continued throughout the study. Tumor volume was measured biweekly using a plethysmometer. Data are expressed as the mean tumor volume \pm the SE. Animals were sacrificed by CO₂ inhalation, serum collected by cardiac puncture and tumors were excised and fixed in Streck STF fixative for 12–18 h at 4°C (Streck Laboratory, Omaha, NE). All animal treatment protocols were reviewed by and were in compliance with Pharmacia's Institutional Animal Care and Use Committee.

Tumor PGE₂. Tumor tissue was weighed at necropsy, snap frozen, and stored at -70°C until use. Frozen tissues were homogenized using a Polytron (Brinkman) in 100% methanol, centrifuged, and the supernatants were collected, dried under a stream of nitrogen, resuspended in EIA buffer, and subsequently assayed for PGE₂ by EIA (Cayman Chemical, Ann Arbor, MI).

Serum Thromboxane B₂. Blood was collected at the end of the study by cardiac puncture, incubated at 37°C for 30 min, and centrifuged at 6000 rpm. Serum was diluted into EIA buffer, and TXB₂ was measured by EIA (Cayman Chemical).

Immunohistochemical Methods. For proliferation (BrdUrd) and apoptosis (TUNEL) analysis, the tumor tissues were dehydrated and embedded in paraffin. Sections were dewaxed, rehydrated through ethanols, and blocked for endogenous peroxidase using routine procedures. BrdUrd containing nuclei were labeled with a biotin-conjugated mouse anti-BrdUrd antibody according to procedures provided by vendor (Zymed Laboratories, South San Francisco, CA). Apoptotic nuclei were labeled with biotin dUTP using terminal deoxynucleotidyl transferase enzyme reagents for 90 min at 37°C (Trevigen, Inc., Gaithersburg, MD) on sections pretreated with 0.25% Triton X-100 in TBS at 50°C for 20 min. Anti-BrdUrd and TUNEL biotin labels were visualized with streptavidin peroxidase and 3,3'-diaminobenzidine (Dako Corp., Carpinteria, CA) followed by hematoxylin counterstain.

For COX immunohistochemistry, paraffin sections were dewaxed, rehydrated, peroxidase blocked, and antigen retrieved at pH 6.0 for 20 min at 95°C (Dako Corp.). Rabbit polyclonal COX-2 antibody (Cayman Chemical) was applied in TBS containing 2% BSA (TBS-BSA) for overnight at 4–8°C. Primary antibodies were detected with Envision reagent (Dako Corp.) followed by peroxidase-diaminobenzidine or peroxidase-aminoethylcarbazole visualization and hematoxylin counterstain. Stained sections were mounted in Permount.

For immunofluorescence expression analysis, tumor tissue slices were imbibed in 15% sucrose in TBS for 6–7 h followed by 30% sucrose in TBS for 24 h before freezing in ornithine carbamyl transferase with liquid nitrogen-cooled isopentane. Endothelial cell labeling used a rat antimouse CD31 monoclonal antibody (Chemicon) followed by biotin donkey antirat IgG (Jackson Labs, West Grove, PA) and streptavidin-Alexa 488 (Molecular Probes, Eugene, OR) in TBS +0.5% Tween 20 (TBST). COX-2 labeling was performed with a rabbit polyclonal COX-2 antibody (Cayman Chemical) in TBS-BSA for 2 h followed by biotin swine antirabbit IgG (Dako Corp.) and streptavidin-Alexa 594 and 4',6-diamidino-2-phenylindole (Molecular Probes) in TBST. Colocalization labeling combined both procedures on the same section. Sections were mounted in Prolong Antifade.

Image Acquisition and Analysis. Digital images were collected on an Olympus AX-70 microscope equipped with digital camera, liquid crystal tunable filter, and computer as described previously (36). Fluorescence images were acquired using excitation filters, and the tunable liquid crystal filter as the emission filter at the appropriate wavelengths for each fluorophore. Colocalization images were corrected for cross-talk between fluorophores using routine image processing methods.

Tumor cell proliferation and apoptosis were measured by computer-based color image analysis using a method based on differential absorption (37) to count total tumor cell nuclei and stained nuclei in image fields of tumor section. For a given tumor, 10–15 images were analyzed, and the average proliferation or apoptotic index from these images represented the value for that animal. The individual tumor values were then averaged for each treatment group and expressed as the percentage of total cells with SE. This analysis was performed on tumor regions that contained healthy proliferating tissue typically found at the tumor margins. Necrotic tissue lying at the center of each tumor was excluded from analysis.

Tumor stromal cell proliferation and apoptosis were measured by visually counting the number of BrdUrd or TUNEL stained and unstained stromal cell nuclei in microvessel structures in a $\times 40$ high power field in two to three sections of tumor. For a given animal, 15–20 microvessels consisting of 100–150 endothelial cells were examined. The endothelial cell proliferation or apoptotic index for a given animal was obtained by dividing the total number of labeled nuclei by the total cell count. Indices from animals in the same treatment group were averaged and expressed as the mean with SE.

RESULTS

Development of a Xenograft Model to Study the Role of COX-2 in Cancer. To study the role of COX-2 and prostaglandins in tumor growth, several cancer cell lines were implanted into mice and, after established tumor growth, were analyzed for known features of human cancer. Of nine cell lines studied *in vivo*, including LNCaP, Lewis lung, and B16F10, all of the tumors cells expressed COX-1 but not COX-2, with the exception of COX-2 expression in the angiogenic vasculature. In contrast, primary cultures of 1483 cells, derived from a human HNSCC, expressed COX-2 *in vitro*, were tumorigenic, and maintained COX-2 expression and activity *in vivo* (Fig. 1). 1483 tumors maintained very high levels of PGE₂ compared with the normal left paw. Interestingly, the expression pattern of COX-2 was similar to that observed in human head and neck cancer (Refs. 22, 25; Fig. 1, A and B). In tumors, clusters of neoplastic epithelial cells having moderate to strong COX-2 expression and stromal cells with considerably lower but significant levels of COX-2 were observed throughout the tumors. (Fig. 1C, *green color*). Endothelial cells were identified using anti-CD31 antibody (Fig. 1D, *red color*). Image overlays of CD31 and COX-2-positive cells in the same section confirmed the presence of COX-2 in the microvasculature (Fig. 1E, *orange color*).

Celecoxib Inhibits 1483 Tumor Growth and PGE₂ Production *in Vivo*. Because the 1483 tumor cells express COX-2 *in vivo* similar to that observed in human cancer samples, the role of COX activity and PGE₂ production in 1483 tumor growth was evaluated pharmacologically with selective inhibitors for COX-1 and COX-2. Typi-

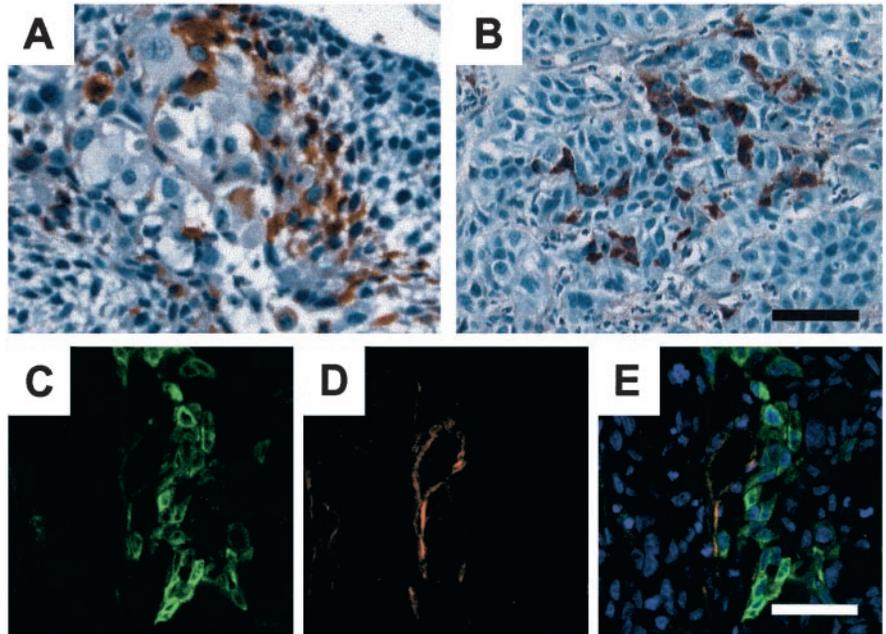


Fig. 1. COX-2 expression in (A) a human head and neck cancer and (B) 1483 human head and neck xenograft tumor. COX-2 expression was observed in clusters of neoplastic epithelial cells throughout the tumor. COX-2 expression was also observed in some but not all stromal cells. Colocalization of (C) COX-2 and endothelial cell marker (D) CD31 in 1483 tumor. Image overlay of (E) CD31 and COX-2 illustrate coexpression (orange) in some endothelial cells. Other stromal cells negative for CD31 also expressed COX-2 (data not shown). On the basis of fluorescence intensity, COX-2 expression in endothelial cells was ~10-fold lower than in tumor cells. Bar = 25 μ m.

cally, animals in the vehicle-treated control groups developed tumors ranging from 0.62 to 1.46 ml in ~27 days (Fig. 2). Treatment starting at 0.1 ml with celecoxib, a COX-2 inhibitor, reduced tumor growth in a dose-dependent manner. At day 27, celecoxib at 40 and 160 ppm inhibited tumor growth by 57 and 78% of vehicle controls, respectively (Fig. 2A). This inhibition of tumor growth was associated with a 62 and 91% decrease in the levels of tumor PGE₂ (Fig. 2A). In contrast, celecoxib at the low dose of 10 ppm did not inhibit tumor growth and only decreased tumor PGE₂ levels by a modest 35% (Fig. 2A). The role of COX-1 in tumor growth was examined with a selective COX-1 inhibitor, SC-560. At 20 ppm, a dose known to inhibit COX-1, but not COX-2 enzyme SC-560, had no effect on tumor growth or tumor PGE₂ levels (Fig. 2B). In contrast, tumor growth was inhibited by 81 and 91% when celecoxib was administered at doses of 160 and 1600 ppm, respectively (Fig. 2B). Furthermore, doses of 160 and 1600 ppm of celecoxib inhibited tumor PGE₂ by 67 and 83%, respectively (Fig. 2B). Thus, inhibition of COX-2 by celecoxib and not COX-1 resulted in tumor inhibition. Also, celecoxib treatment did not alter COX-1 or COX-2 protein levels measured by Western blot analyses (data not shown).

In Vivo Selectivity of COX Inhibitors. *In vivo* selectivity of each COX inhibitor was evaluated in serum from treated animals by immunoassaying TXB₂ levels, the degradation product of TxA₂ formed in the platelet by COX-1. Inhibition of tumor growth and PGE₂ by celecoxib was not associated with platelet COX-1 at any dose. SC-560, however, inhibited COX-1-derived TXB₂ by 84% compared with vehicle controls and normal nontumor bearing mice (normal, Fig. 3). These data indicate that celecoxib selectively inhibited COX-2 activity and PGE₂ production in 1483 tumors and that inhibition of PGE₂ correlated well with the inhibition of tumor growth.

1483 Tumor Growth Is PGE₂ Dependent. The role of PGE₂ in the growth of 1483 tumors was additionally examined in tumor-bearing mice treated with a PGE₂-neutralizing mouse monoclonal antibody (2B5; Ref. 38). After a dosing regimen previously described (39), 2B5 antibody treatment resulted in a similar inhibition of tumor growth as observed with celecoxib (93 *versus* 84% of vehicle treated control; Fig. 4). The MOPC31 IgG isotype control did not reduce tumor growth. Although the 2B5 antibody was an effective treatment for tumor control, dosing had to be discontinued at day 21 because of

gastrointestinal toxicity, leading to body weight loss and death. Thus, the treatment with the COX-2 inhibitor celecoxib was able to inhibit COX-2 derived PGE₂ while sparing constitutive COX-1-derived PGE₂ necessary for normal gastrointestinal homeostasis.

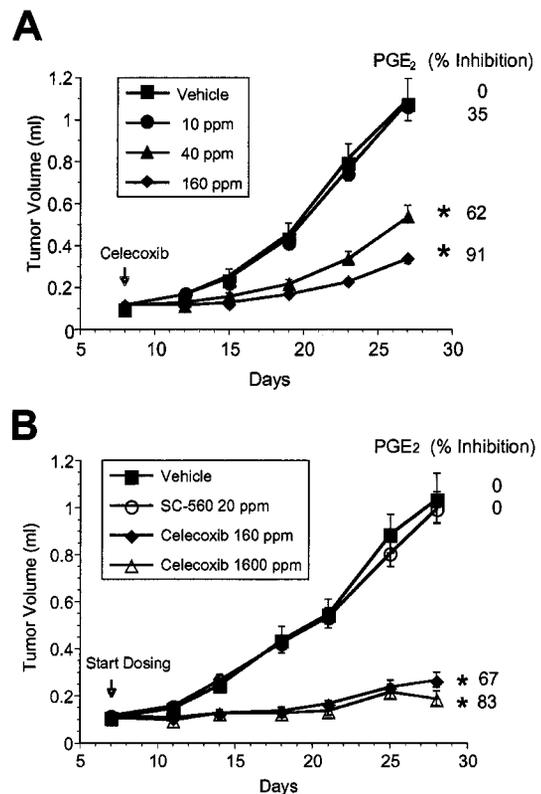


Fig. 2. Effect of cyclooxygenase inhibitors on 1483 xenograft tumor growth and PGE₂ production. Vehicle-treated 1483 tumors exhibited a robust exponential growth starting at day 7. Celecoxib treatment at 10–160 ppm (A) and 1600 ppm (B) produced a dose-dependent inhibition in growth and PGE₂ content after 28 days. B, a selective COX-1 inhibitor, SC-560, at a dose that significantly inhibits serum platelet COX-1 but not COX-2 (20 ppm) had no effect on tumor growth. Celecoxib dosed dependently inhibited this production, whereas SC-560 had no effect. Data represent the mean \pm SE (n = 8 mice/group); *, P < 0.05 compared with vehicle treated.

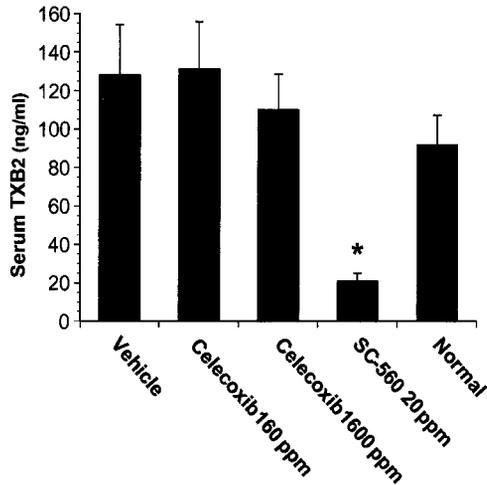


Fig. 3. Effect of cyclooxygenase inhibitors on platelet COX-1-derived serum thromboxane production as measured by standard EIA. Serum thromboxane production by platelet COX-1 was markedly inhibited by SC-560 but unaffected by celecoxib. These data illustrate the selectivity of celecoxib for COX-2 in these studies and illustrate the important role of COX-2 compared with COX-1 in 1483 tumor growth. Data represent the mean \pm SE ($n = 8$ mice/group); *, $P < 0.05$ compared with vehicle treated.

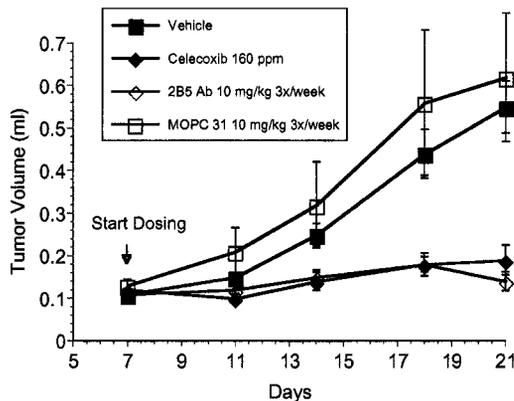


Fig. 4. Effect of anti-PGE₂-neutralizing antibody on 1483 tumor growth. i.p. injections of 2B5 mouse anti-PGE₂ antibody, 10 mg/kg, 3 times/week, was as efficacious as celecoxib (160 ppm) in inhibiting 1483 tumor growth. Continued dosing, however, was toxic and produced GI lesions and death in all animals after 14 days of dosing. Control IgG, MOPC31, at equivalent doses had no effect on tumor growth or animal health. Data represent the mean \pm SE ($n = 8$ mice/group); *, $P < 0.05$ compared with vehicle treated.

In Vivo Inhibition of COX-2-derived PGE₂ Results in Apoptosis and Reduced Proliferation in 1483 Tumors. The effects of COX-2 inhibition on tumor epithelial and endothelial cell proliferation and apoptosis were examined *in vivo*. Celecoxib (160 and 1600 ppm) or

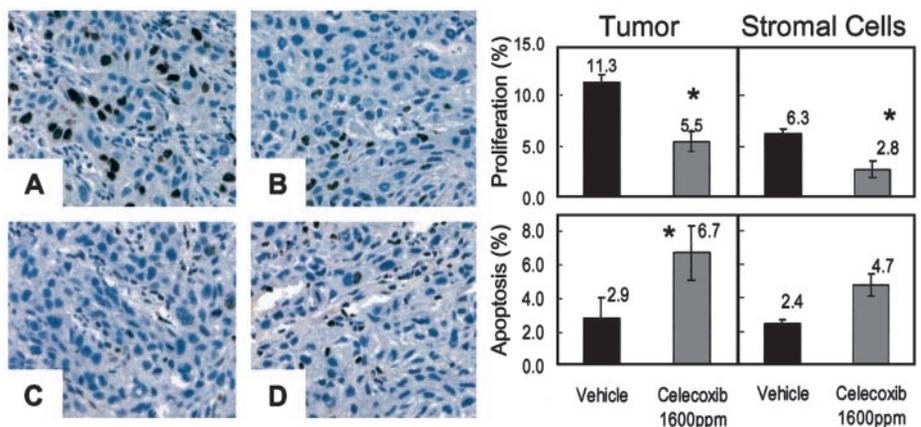
SC-560 (20 ppm) was administered in the food to 1483 tumor-bearing (0.4 ml) mice for a total of 12 days. On day 12, compound and vehicle treated mice were injected i.p. with BrdUrd and sacrificed 2 h later. BrdUrd incorporation, an index marker of cellular proliferation, was determined in both the tumor cells and associated stromal cells (Fig. 5). Celecoxib treatment inhibited tumor and stromal cell proliferation by 51 and 56%, respectively, corresponding to a decrease in the proliferation index from 11.3 to 5.5% and 6.3 to 2.8%, respectively (Fig. 5). Similar effects on proliferation were observed on tumors treated with 160 ppm celecoxib (data not shown). Apoptosis, measured by TUNEL staining, was increased by celecoxib in the tumor cells by 57%, increasing the apoptosis index from 2.9 to 6.7%. The stromal cell apoptotic index increased from 2.4 to 4.7%, an increase of 49% (Fig. 5). Inhibition of COX-1 by SC-560, however, did not significantly impact either of these parameters (data not shown).

Effect of COX-2-derived PGE₂ on Normal Proliferating Epithelium. To address the role of COX-2 in normal proliferating cells, a COX-2 inhibitor (celecoxib), a nonselective NSAID (indomethacin), and a cytotoxic agent (5-FU) were tested in their ability to inhibit proliferation of ileum epithelium in the crypts. Vehicle, celecoxib (160 and 1600 ppm), indomethacin (2 mg/kg/day), and 5-FU (40 mg/kg/day) were administered for 4 days in nude mice, and 24 h after the last administration of 5-FU, mice were injected with BrdUrd and sacrificed 2 h later. Proliferation of normal cells (as represented by ileum crypt BrdUrd incorporation) was not affected by either dose of celecoxib, whereas proliferation of intestinal epithelium in animals treated with indomethacin or 5-FU was inhibited by 36 and 77%, respectively (Fig. 6).

DISCUSSION

Prostaglandins have long been considered important in tumor growth and development. However, reducing prostaglandin levels within tumors by use of nonselective NSAIDs in conjunction with a chemotherapeutic or radiation therapeutic regimen would likely be limited because of toxicity that could occur to proliferating tissues such as the GI tract. Discovery of distinct isoforms of cyclooxygenase led to the notion that selective inhibition of COX-2 would avoid the gastric toxicity associated with inhibition of both isoforms. The observation that COX-2 is highly expressed in a majority of human tumors suggests that COX-2 inhibition may be a useful therapeutic mechanism for inhibiting tumor progression and growth of human cancer (22, 40–44). A role for COX-2 in cancer is additionally supported by the observation that genetic deletion of host COX-2 results in reduced tumor generation and growth rates (34) and that tissue-specific overexpression of COX-2 leads to tumor formation in transgenic mice (14). These data additionally support the hypothesis

Fig. 5. Effect of celecoxib treatment on tumor and stromal cell proliferation and apoptosis *in vivo*. Using BrdUrd immunohistochemistry (A and B) to assess proliferation, celecoxib consistently inhibited proliferation in tumor cells (upper right graph) and stromal cells (upper left graph) by 50–60% after 12 days of treatment (*, $P < 0.0002$ and $P < 0.02$, respectively). Using TUNEL to assay apoptosis in tumor sections (C and D), celecoxib also induced a consistent 2–3-fold increase in tumor cell (lower right graph; *, $P < 0.01$) and stromal cell (lower left graph) apoptosis. SC-560, the selective COX-1 inhibitor had no effect on either parameter (data not shown).



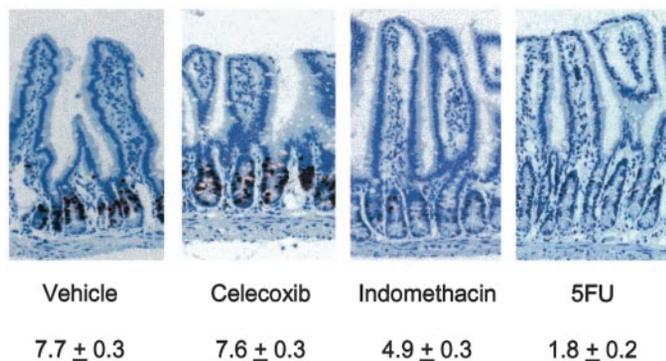


Fig. 6. Effect of celecoxib, indomethacin, and 5-FU on epithelial cell proliferation in the ileum of nontumor bearing mice. After 4 days of treatment, the number of BrdUrd-labeled cells per crypt were counted by visual microscopy, and the mean and SE for each treatment group (6 animals) were determined. GI proliferation was not affected by celecoxib (1600 ppm) after 4 days or even after 56 days of treatment (data not shown). Indomethacin, (2 mg/day) and 5-FU, (40 mg/day × 4 days), however, inhibited GI proliferation by 36 and 77%, respectively.

that inhibiting intratumoral, COX-2-derived prostaglandin synthesis could be an effective anticancer therapy. This hypothesis has become testable with the development of COX-2 inhibitors such as celecoxib. Early studies on cancer prevention have proven these inhibitors are very effective in a number of preclinical cancer models, particularly colon (13, 45), breast (12), bladder (11), and skin (46), (47) and celecoxib has recently been approved for use in patients with familial adenomatous polyposis. Preclinical tumor models have also provided supportive evidence (9, 16, 29, 48). However most of these models were murine allografts or xenografts and correlation of tumor control with demonstrable prostaglandin reduction has been inconsistent. This may be attributable to the restricted expression of COX-2 to the neovascular and stromal cells in many of these models. In contrast, the COX-2 expression patterns observed in human tumors include abundant clusters of intensely COX-2-positive tumor cells and high levels of COX-2-derived prostaglandin, particularly PGE₂. These murine models have supported the hypothesis that COX-2 activity is important in the development and maintenance of tumor vasculature (16, 29, 34, 49, 50). However, as models for predicting the efficacy of COX-2 inhibitors in the clinic, they are limited in that they do not sufficiently mimic COX-2 expression of human cancer.

With few exceptions (including the 1483 model discussed in this manuscript), our attempts to create or find a murine model that expresses COX-2 similarly to human solid tumors have been unsuccessful. Stable HT-29 transfectants that produced human COX-2 behind a constitutive mouse mammary tumor virus promoter did not form tumors. Other human or mouse tumor cell lines that expressed COX-2 *in vitro* lost this expression after implantation into mice. In contrast, the 1483 cell line formed rapidly growing tumors with a pathology that resembled human head and neck cancer in terms of overall histology, COX-2 expression, and prostaglandin content. Using this model, we have demonstrated that reduced intratumoral PGE₂ correlates with reduced tumor growth, reduced tumor and endothelial cell proliferation, and cell survival. We have shown pharmacological evidence that PGE₂ is derived predominantly from COX-2 activity because PGE₂ levels are significantly decreased by celecoxib treatment at COX-2 inhibitory doses. Although genetic models, (*i.e.*, knockout mice) have implicated both COX-1 and COX-2 in tumor formation and growth (51), our results with a COX-1 selective inhibitor, SC-560, suggest that COX-1 does not play a significant role in tumor growth or overall prostaglandin levels in this model. A key finding of our study is the central importance of COX-2-derived PGE₂ in driving tumor growth as revealed by the antitumor activity of a

neutralizing anti-PGE₂ antibody that mimicked celecoxib therapy. The advantage of celecoxib, however, is its lack of GI toxicity. These findings further suggest that celecoxib may provide a means for safe and effective anticancer therapy and clearly link this antitumor efficacy to inhibition of COX-2 enzymatic activity and PGE₂ inhibition. Clinical trials with celecoxib in human cancer will be required to prove clinical benefit.

REFERENCES

- Koki, A. T., Leahy, K. M., and Masferrer, J. L. Potential utility of COX-2 inhibitors in chemoprevention and chemotherapy. *Expert. Opin. Investig. Drugs*, 8: 1623–1638, 1999.
- Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., and Isakson, P. Distribution of COX-1 and COX-2 in normal and inflamed tissues. *Adv. Exp. Med. Biol.*, 400A: 167–170, 1997.
- Yoshimatsu, K., Golijanin, D., Paty, P. B., Soslow, R. A., Jakobsson, P. J., DeLellis, R. A., Subbaramaiah, K., and Dannenberg, A. J. Inducible microsomal prostaglandin E synthase is overexpressed in colorectal adenomas and cancer. *Clin. Cancer Res.*, 7: 3971–3976, 2001.
- Fu, J. Y., Masferrer, J. L., Seibert, K., Raz, A., and Needleman, P. The induction and suppression of prostaglandin H₂ synthase (cyclooxygenase) in human monocytes. *J. Biol. Chem.*, 265: 16737–16740, 1990.
- DuBois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B., and Lipsky, P. E. Cyclooxygenase in biology and disease. *FASEB J.*, 12: 1063–1073, 1998.
- Thun, M. J. NSAID use and decreased risk of gastrointestinal cancers. *Gastroenterol. Clin. N. Am.*, 25: 333–348, 1996.
- Funkhouser, E. M., and Sharp, G. B. Aspirin and reduced risk of esophageal carcinoma. *Cancer (Phila.)*, 76: 1116–1119, 1995.
- Thun, M. J., Nambodiri, M. M., Calle, E. E., Flanders, W. D., and Heath, C. W., Jr. Aspirin use and risk of fatal cancer. *Cancer Res.*, 53: 1322–1327, 1993.
- Jacoby, R. F., Seibert, K., Cole, C. E., Kelloff, G., and Lubet, R. A. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res.*, 60: 5040–5044, 2000.
- Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. Suppression of intestinal polyposis in Apc Δ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell*, 87: 803–809, 1996.
- Grubbs, C. J., Lubet, R. A., Koki, A. T., Leahy, K. M., Masferrer, J. L., Steele, V. E., Kelloff, G. J., Hill, D. L., and Seibert, K. Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F₁ mice and female Fischer-344 rats. *Cancer Res.*, 60: 5599–5602, 2000.
- Harris, R. E., Alshafie, G. A., Abou-Issa, H., and Seibert, K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. *Cancer Res.*, 60: 2101–2103, 2000.
- Reddy, B. S., Hirose, Y., Lubet, R., Steele, V., Kelloff, G., Paulson, S., Seibert, K., and Rao, C. V. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res.*, 293–297, 2000.
- Liu, C. H., Chang, S. H., Narko, K., Trifan, O. C., Wu, M. T., Smith, E., Haudenschild, C., Lane, T. F., and Hla, T. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J. Biol. Chem.*, 276: 18563–18569, 2001.
- Fosslien, E. Biochemistry of cyclooxygenase (COX)-2 inhibitors and molecular pathology of COX-2 in neoplasia. *Crit. Rev. Clin. Lab. Sci.*, 37: 431–502, 2000.
- Masferrer, J. L., Leahy, K. M., Koki, A. T., Zweifel, B. S., Settle, S. L., Woerner, B. M., Edwards, D. A., Flickinger, A. G., Moore, R. J., and Seibert, K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.*, 60: 1306–1311, 2000.
- Balch, C. M., Dougherty, P. A., and Tilden, A. B. Excessive prostaglandin E₂ production by suppressor monocytes in head and neck cancer patients. *Ann. Surg.*, 196: 645–650, 1982.
- Bennett, A., Tacca, M. D., Stamford, I. F., and Zebro, T. Prostaglandins from tumours of human large bowel. *Br. J. Cancer*, 6: 881–884, 1977.
- Smith, B. J., Wills, M. R., and Savory, J. Prostaglandins and cancer. *Ann. Clin. Lab. Sci.*, 13: 359–365, 1983.
- Seyberth, H. W., Segre, G. V., Morgan, J. L., Sweetman, B. J., Potts, J. T., Jr., and Oates, J. A. Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. *N. Engl. J. Med.*, 293: 1278–1283, 1975.
- Taketo, M. M. Cyclooxygenase-2 inhibitors in tumorigenesis (Part II). *J. Natl. Cancer Inst. (Bethesda)*, 90: 1609–1620, 1998.
- Chan, G., Boyle, J. O., Yang, E. K., Zhang, F., Sacks, P. G., Shah, J. P., Edelstein, D., Soslow, R. A., Koki, A. T., Woerner, B. M., Masferrer, J. L., and Dannenberg, A. J. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. *Cancer Res.*, 59: 991–994, 1999.
- Jung, T. T., Berlinger, N. T., and Juhn, S. K. Prostaglandins in squamous cell carcinoma of the head and neck: a preliminary study. *Laryngoscope*, 95: 307–312, 1985.
- Mestre, J. R., Chan, G., Zhang, F., Yang, E. K., Sacks, P. G., Boyle, J. O., Shah, J. P., Edelstein, D., Subbaramaiah, K., and Dannenberg, A. J. Inhibition of cyclooxygenase-2 expression. An approach to preventing head and neck cancer. *Ann. N. Y. Acad. Sci.*, 889: 62–71, 1999.

25. Zimmermann, K. C., Sarbia, M., Weber, A. A., Borchard, F., Gabbert, H. E., and Schror, K. Cyclooxygenase-2 expression in human esophageal carcinoma. *Cancer Res.*, 59: 198–204, 1999.
26. Sumitani, K., Kamijo, R., Toyoshima, T., Nakanishi, Y., Takizawa, K., Hatori, M., and Nagumo, M. Specific inhibition of cyclooxygenase-2 results in inhibition of proliferation of oral cancer cell lines via suppression of prostaglandin E₂ production. *J. Oral Pathol. Med.*, 31: 41–47, 2001.
27. Form, D. M., and Auerbach, R. PGE₂ and angiogenesis. *Proc. Soc. Exp. Biol. Med.*, 172: 214–218, 1983.
28. Hanahan, D., and Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*, 86: 353–364, 1996.
29. Leahy, K. M., Ornberg, R. L., Wang, Y., Zweifel, B. S., Koki, A. T., and Masferrer, J. L. Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in angiogenic endothelial cells *in vivo*. *Cancer Res.*, 62: 625–631, 2002.
30. Hsu, A. L., Ching, T. T., Wang, D. S., Song, X., Rangnekar, V. M., and Chen, C. S. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. *J. Biol. Chem.*, 275: 11397–11403, 2000.
31. Song, X., Lin, H. P., Johnson, A. J., Tseng, P. H., Yang, Y. T., Kulp, S. K., and Chen, C. S. Cyclooxygenase-2, player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostate cancer cells. *J. Natl. Cancer Inst. (Bethesda)*, 94: 585–591, 2002.
32. Zhou, X. M., Wong, B. C., Fan, X. M., Zhang, H. B., Lin, M. C., Kung, H. F., Fan, D. M., and Lam, S. K. Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. *Carcinogenesis (Lond.)*, 22: 1393–1397, 2001.
33. Masferrer, J. Approach to angiogenesis inhibition based on cyclooxygenase-2. *Cancer J.*, 7 (Suppl. 3): S144–S150, 2001.
34. Williams, C. S., Tsujii, M., Reese, J., Dey, S. K., and DuBois, R. N. Host cyclooxygenase-2 modulates carcinoma growth. *J. Clin. Investig.*, 105: 1589–1594, 2000.
35. Sacks, P. G., Parnes, S. M., Gallick, G. E., Mansouri, Z., Lichtner, R., Satya-Prakash, K. L., Pathak, S., and Parsons, D. F. Establishment and characterization of two new squamous cell carcinoma cell lines derived from tumors of the head and neck. *Cancer Res.*, 48: 2858–2866, 1988.
36. Ornberg, R. L., Woerner, B. M., and Edwards, D. A. Analysis of stained objects in histological sections by spectral imaging and differential absorption. *J. Histochem. Cytochem.*, 47: 1307–1314, 1999.
37. Ornberg, R. L. Proliferation and apoptosis measurements by color image analysis based on differential absorption. *J. Histochem. Cytochem.*, 49: 1059–1060, 2001.
38. Mnich, S. J., Veenhuizen, A. W., Monahan, J. B., Sheehan, K. C., Lynch, K. R., Isakson, P. C., and Portanova, J. P. Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E₂. *J. Immunol.*, 155: 4437–4444, 1995.
39. Stolina, M., Sharma, S., Lin, Y., Dohadwala, M., Gardner, B., Luo, J., Zhu, L., Kronenberg, M., Miller, P. W., Portanova, J., Lee, J. C., and Dubinett, S. M. Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J. Immunol.*, 164: 361–370, 2000.
40. Khan, K. N., Masferrer, J. L., Woerner, B. M., Soslow, R., and Koki, A. T. Enhanced cyclooxygenase-2 expression in sporadic and familial adenomatous polyposis of the human colon. *Scand. J. Gastroenterol.*, 36: 865–869, 2001.
41. Tucker, O. N., Dannenberg, A. J., Yang, E. K., Zhang, F., Teng, L., Daly, J. M., Soslow, R. A., Masferrer, J. L., Woerner, B. M., Koki, A. T., and Fahey, T. J., III. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. *Cancer Res.*, 59: 987–990, 1999.
42. Kulkarni, S., Rader, J. S., Zhang, F., Liapis, H., Koki, A. T., Masferrer, J. L., Subbaramaiah, K., and Dannenberg, A. J. Cyclooxygenase-2 is overexpressed in human cervical cancer. *Clin. Cancer Res.*, 7: 429–434, 2001.
43. Mohammed, S. I., Knapp, D. W., Bostwick, D. G., Foster, R. S., Khan, K. N., Masferrer, J. L., Woerner, B. M., Snyder, P. W., and Koki, A. T. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. *Cancer Res.*, 59: 5647–5650, 1999.
44. Soslow, R. A., Dannenberg, A. J., Rush, D., Woerner, B. M., Khan, K. N., Masferrer, J., and Koki, A. T. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer (Phila.)*, 89: 2637–2645, 2000.
45. Williams, C. S., Sheng, H., Brockman, J. A., Armandla, R., Shao, J., Washington, M. K., Elkahoun, A. G., and DuBois, R. N. A cyclooxygenase-2 inhibitor (SC-58125) blocks growth of established human colon cancer xenografts. *Neoplasia*, 3: 428–436, 2001.
46. Fischer, S. M., Lo, H. H., Gordon, G. B., Seibert, K., Kelloff, G., Lubet, R. A., and Conti, C. J. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol. Carcinog.*, 25: 231–240, 1999.
47. Pentland, A. P., Schoggins, J. W., Scott, G. A., Khan, K. N., and Han, R. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis (Lond.)*, 20: 1939–1944, 1999.
48. Sheng, H., Shao, J., Kirkland, S. C., Isakson, P., Coffey, R. J., Morrow, J., Beauchamp, R. D., and DuBois, R. N. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J. Clin. Investig.*, 99: 2254–2259, 1997.
49. Leahy, K. M., Koki, A. T., and Masferrer, J. L. Role of cyclooxygenases in angiogenesis. *Curr. Med. Chem.*, 7: 1163–1170, 2000.
50. Masferrer, J. L., Koki, A., and Seibert, K. COX-2 inhibitors. A new class of antiangiogenic agents. *Ann. N. Y. Acad. Sci.*, 889: 84–86, 1999.
51. Chulada, P. C., Thompson, M. B., Mahler, J. F., Doyle, C. M., Gaul, B. W., Lee, C., Tiano, H. F., Morham, S. G., Smithies, O., and Langenbach, R. Genetic disruption of PtgS-1, as well as PtgS-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res.*, 60: 4705–4708, 2000.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Direct Evidence for a Role of Cyclooxygenase 2-derived Prostaglandin E₂ in Human Head and Neck Xenograft Tumors

Ben S. Zweifel, Thomas W. Davis, Richard L. Ornberg, et al.

Cancer Res 2002;62:6706-6711.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/62/22/6706>

Cited articles This article cites 48 articles, 20 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/62/22/6706.full#ref-list-1>

Citing articles This article has been cited by 32 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/62/22/6706.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/62/22/6706>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.