Cyclooxygenase-2 Inhibitors in Tumorigenesis (Part II)

Makoto M. Taketo

The rate-limiting step in arachidonate metabolism is mediated by enzymes known as cyclooxygenases (COXs). These enzymes catalyze the biosynthesis of prostaglandin H₂, the precursor of molecules such as prostaglandins, prostacyclin, and thromboxanes. The COX enzyme family consists of the classical COX-1 enzyme, which is constitutively expressed in many tissues, and a second isozyme, i.e., COX-2, which is induced by various stimuli, such as mitogens and cytokines, and is involved in many inflammatory reactions. Because nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both COX-1 and COX-2, these drugs also cause unwanted side effects, exemplified by gastrointestinal bleeding. Accumulating evidence indicates that NSAIDs can reduce the incidence of colorectal cancers in human and experimental animals and can reduce the number and size of polyps in patients with familial adenomatous polyposis. This Part II (of a two-part review) focuses on the growing clinical and experimental evidence that NSAIDS and COX-2 inhibitors can influence the risk of colon (and possibly of other) cancers. [J Natl Cancer Inst 1998;90:1609–20]

Cyclooxygenase (COX) is the key enzyme in arachidonate metabolism and catalyzes the biosynthesis of prostaglandin H₂, the precursor for prostanoids. In addition to COX-1, which is constitutively expressed in many tissues, another isozyme (COX-2) was identified in 1991. COX-2 is induced in many inflammatory reactions. Because nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both COX-1 and COX-2, these drugs also cause unwanted side effects exemplified by gastrointestinal bleeding. In Part I of this review (see “Notes” section), I summarized the biochemistry and pharmacology of COXs and their inhibitors: arachidonate metabolism and COXs, the regulation of prostaglandin synthesis, NSAIDs and the inhibition of COXs, COX-2 selective inhibitors and the inhibition of COX isozymes, and the structural basis of functional differences between COX-1 and COX-2.

In Part II of this review, I will first describe earlier data concerning the effects of NSAIDs on colorectal tumors and will then present an overview of research on the influence of COX-2 and its inhibitors on cancers. I will focus on the role of these molecules in colorectal cancer and its animal models, with some extension to other types of cancer, and discuss the clinical relevance of these compounds: Accumulating evidence indicates that NSAIDs can reduce the incidence of colorectal cancers in human and experimental animals and can reduce the number and size of polyps in patients with familial adenomatous polyposis (FAP). Recently, evidence has been presented that COX-2 is induced in human colorectal cancers and in the polyps of mouse FAP models. When the COX-2 gene is inactivated in FAP-model mice, both the number and the size of polyps are reduced dramatically. In addition, selective inhibitors of COX-2 cause results similar to those caused by COX-2 gene knockout mutations. These genetic and pharmacologic data open up the possibility of effectively treating human FAP and various human cancers with COX-2 selective inhibitors, a new class of NSAIDs.

Colorectal Cancer and Prostaglandins

Colorectal cancer is one of the leading causes of cancer mortality in the United States and other developed countries (1). There is strong evidence suggesting that virtually all colorectal adenocarcinomas arise within pre-existing adenomas or areas of high-grade dysplasia (2). The risk of cancer increases as an adenoma becomes larger, has a greater villous component, or contains more high-grade dysplasia. In exceptional cases, however, some carcinomas are likely to develop in small and highly dysplastic flat adenomas (3).

As in other systems such as skin cancer (4), colorectal carcinogenesis has been shown to involve many genetics steps (5–7). The triggering events in colorectal carcinogenesis were identified through molecular genetic studies of hereditary forms of the diseases. One such hereditary condition, FAP, was found to be caused by mutations in the adenomatous polyposis coli (APC) gene, whereas hereditary nonpolyposis colorectal cancer (HNPPC) was found to be due to mutations in one of several DNA mismatch repair genes (7). However, details of the molecular processes that occur in the adenoma–carcinoma sequence after biallelic inactivation of APC or the DNA repair genes are yet to be investigated.

On the other hand, circumstantial evidence for possible involvement of COXs in colorectal cancer has been derived from pharmacologic analyses of prostaglandins. Various animal and human tumor tissues, including human colon cancer, have been reported to contain high concentrations of prostaglandins (8–10). This relationship of neoplastic tumors to increased levels of
prostaglandins provided the rationale for earlier use of NSAIDs as potential chemoprevention agents. These drugs inhibit endogenous prostaglandin synthesis, which plays a role in the control of neoplastic and non-neoplastic cell proliferation and of immune functions (11–14). Three independent lines of research have provided support for this approach in numerous published reports of experiments on animal models, epidemiologic studies, and clinical trials on FAP patients.

**Effect of NSAIDs on Colon Cancer: Earlier Studies on Animal Models**

In experimental animals transplanted with various tumors [e.g., mast cell ascites tumors (15,16), fibrosarcoma (17), and colon adenocarcinoma (18)], indomethacin, aspirin, and piroxicam were shown to reduce tumor growth. With the use of chemical carcinogen-induced rat and mouse tumor models for colorectal cancer, the NSAIDs indomethacin, meclofenamate, piroxicam, sulindac, and aspirin were shown to decrease the incidence, multiplicity, and/or size of tumors in rats or mice (Table 1). Because most such results are also discussed in the reviews by DuBois et al. (19) and by Levy (20), I have listed these reports in Table 1 and summarized them only briefly here. These studies used N-methyl-N-nitrosourea, 1,2-dimethylhydrazine (DMH), azoxymethane, or methylazoxymethanol as carcinogens. All of these carcinogens are metabolically activated to form an active carcinogen (21,22). DMH is converted to azoxymethane, which is further metabolized to methylazoxymethanol and then to methyldiazonium ion, whereas dimethylnitrosoamine and related compounds are converted to methyldiazonium ion through a separate pathway. A methyldiazonium ion, once formed, generates a carbonium ion that is responsible for methylation of nucleic acids in animals. In Sprague-Dawley rats, once formed, generates a carbonium ion that is responsible for methylation of nucleic acids in animals. In Sprague-Dawley rats, these reports in Table 1 and summarized them only briefly here.

![Table 1. Autochthonous colon tumors effectively suppressed by nonsteroidal anti-inflammatory drugs (NSAIDs)*](https://academic.oup.com/jnci/article-abstract/90/21/1609/2519718/fig_tab1)

<table>
<thead>
<tr>
<th>Animal and strain (sex)</th>
<th>Carcinogen</th>
<th>NSAID</th>
<th>References and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMH</td>
<td>Indomethacin</td>
<td>Pollard and Luckert, 1982 (108)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMH</td>
<td>Indomethacin</td>
<td>Pollard and Luckert, 1983 (24)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMH</td>
<td>Indomethacin or meclofenamate</td>
<td>Metzger et al., 1984 (109)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMH</td>
<td>Sulindac</td>
<td>Skinner et al., 1991 (110)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMH</td>
<td>Aspirin</td>
<td>Craven and DeRubertis, 1992 (111) (PGE\textsubscript{2} decreased)</td>
</tr>
<tr>
<td>F344 (m)</td>
<td>AOM</td>
<td>Piroxicam and DFMO</td>
<td>Nigro et al., 1986 (112)</td>
</tr>
<tr>
<td>F344 (m)</td>
<td>AOM</td>
<td>Piroxicam</td>
<td>Reddy et al., 1987 (14)</td>
</tr>
<tr>
<td>F344 (m)</td>
<td>AOM</td>
<td>Piroxicam and DFMO</td>
<td>Rao et al., 1991 (113) (8354 or EA, n/e)</td>
</tr>
<tr>
<td>F344 (m)</td>
<td>AOM</td>
<td>Aspirin</td>
<td>Reddy et al., 1993 (114) (PGE\textsubscript{2} decreased)</td>
</tr>
<tr>
<td>F344 (m)</td>
<td>AOM</td>
<td>Sulindac</td>
<td>Rao et al., 1995 (115)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>DMN</td>
<td>Indomethacin</td>
<td>Pollard and Luckert, 1981 (116)</td>
</tr>
<tr>
<td>Donryu (m)</td>
<td>MAM</td>
<td>Indomethacin</td>
<td>Kudo et al., 1980 (117)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>MAM</td>
<td>Indomethacin</td>
<td>Pollard and Luckert, 1983 (24)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>MAM</td>
<td>Piroxicam</td>
<td>Pollard et al., 1983 (118)</td>
</tr>
<tr>
<td>F344 (f)</td>
<td>MNU</td>
<td>Indomethacin</td>
<td>Nairisawa et al., 1981 (119)</td>
</tr>
<tr>
<td>F344 (f)</td>
<td>MNU</td>
<td>Indomethacin</td>
<td>Nairisawa et al., 1982 (120)</td>
</tr>
<tr>
<td>F344 (f)</td>
<td>MNU</td>
<td>Indomethacin</td>
<td>Nairisawa et al., 1983 (121)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>MNU</td>
<td>Indomethacin</td>
<td>Nairisawa et al., 1984 (122)</td>
</tr>
<tr>
<td>SD (m)</td>
<td>MNU</td>
<td>Piroxicam</td>
<td>Pollard and Luckert, 1984 (123)</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/c (f)</td>
<td>DMH</td>
<td>Sulindac</td>
<td>Moorchens et al., 1988 (26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moorchens et al., 1990 (124) (after 11 wk of DMH, n/e)</td>
</tr>
</tbody>
</table>

*SD = Sprague-Dawley; m = male; f = female; DMH = 1,2-dimethylhydrazine; AOM = azoxymethane; DMN = dimethylnitrosoamine; MAM = methylazoxymethanol; MNU = N-methyl-N-nitrosourea; DFMO = 3,4-difluoromethylornithine; 8354 = dehydroepiandrosterone analogue 16a-fluoro-5-androsten-17-one; EA = ellagic acid; n/e = no effects; PGE\textsubscript{2} = prostaglandin E\textsubscript{2}.
treatment caused tumor regression (Table 2) (28). Following these reports, several other studies were published in which similar results were reported, including confirmation of the reversible nature of the polyp regression caused by sulindac (Table 2) (29–31).

To evaluate the effects of sulindac on FAP in a more objective way, several randomized, placebo-controlled, double-blinded studies have been conducted and published subsequently (Table 2). Labayle et al. (32) employed a 4-month crossover method with a 1-month washout period in 10 post-subcolectomy patients. In contrast, the study conducted by Giardiello et al. (33) contained 18 patients seen preoperatively out of 22 subjects. In both studies, the polyps tended to increase in both number and size during the placebo administration or once sulindac was discontinued. Accordingly, sulindac treatment of preoperative FAP patients was not deemed complete enough to replace colectomy, although it may be useful as an adjunct to surgery in postoperative cases (33,34).

One of the problems often faced in sulindac treatment of FAP patients appears to be the severe side effects that are common to NSAIDs, e.g., bleeding and ulceration. Although some patients tolerate sulindac without such problems, its side effects can be serious, even fatal, in others. For this reason, there has been a strong desire by researchers to develop new therapeutic agents, such as selective inhibitors of COX-2, that lack these side effects (see Part I, published in the previous issue of the Journal [Vol. 90, No. 20, October 21]; see also below.)

EPIDEMIOLOGIC STUDIES

Another line of evidence for the inhibition of colorectal tumorigenesis by NSAIDs was obtained by undertaking epidemiologic studies, which were encouraged by the animal model and FAP studies. Numerous retrospective and prospective studies of NSAID use and colon cancer suppression were conducted, as summarized in Table 3. In most of these studies, the relative risk of developing colon cancer was lower in patients (or the sample population) who took aspirin or other NSAIDs. In elaborate studies in which the effect of dosage and/or duration of NSAID intake was investigated, dose-dependent and/or duration-dependent reductions in the relative risk were often found. In some studies, acetaminophen was used as a control and was found to have no association with colon cancer incidence or mortality. One of the reports (35) employed detection of colorectal adenomas, rather than detection of colorectal cancer, as the end point and gave similar results. The exceptions to this conclusion were two studies: one (36) on a small retirement community in California, in which aspirin intake was associated with an increased risk of colorectal cancer, and the other (37) that used data from the randomized Physician’s Health Study. The interpretation of these particular studies remains controversial.

### COX-1 and COX-2 Gene Knockout Mice

Taking advantage of homologous recombination, which can be induced in mouse embryonic stem cells, researchers constructed gene knockout mice that have homozygous inactivation of either the COX-1 or the COX-2 gene (38–40). Morham et al. (38) and Dinchuk et al. (40) reported the phenotypes of the COX-2 gene (Ptgs2) knockout mice. While some of the phenotypic characteristics reported are similar in the two reports, there are some distinct differences as well. In both studies, the homozygous Ptgs2 (−/−) mutants showed renal dysplasia and developed severe nephropathy. However, Morham et al. also found suppurative peritonitis in two of three Ptgs2 (−/−) mice, whereas Dinchuk et al. found cardiac fibrosis in 50% of their “homozygotes.” Surprisingly, both groups reported that the inflammatory responses of the ear to tetraphorbolacetate and to arachidonic acid were not affected in the homozygous mutants. However,
Dinchuk et al. found a striking mitigation of endotoxin-induced hepatocellular cytotoxicity in the homozygotes, and their females were sterile. It appears that active Ptgs2 is not essential for hepatic toxicity, and COX-2 induction takes place later than 1–4 hours after application of the chemicals, when both groups assayed the effects (38, 40).

In contrast, the homozygous COX-1 gene (Ptgs1) knockout mice survived well, without apparent abnormal phenotypes. Surprisingly, these mice showed no gastric pathology and showed males survived well, without apparent abnormal phenotypes. Survival of these mice was not equivalent to the inhibition of COX-1 activity by indomethacin. For example, the residual peroxidase activity of the COX-1–indomethacin complex generates peroxidation products of arachidonic acid, which may be responsible for the ulceration (41).

**COX-2 INDUCTION IN COLORECTAL CANCER AND EFFECTS OF NSAIDS: NEWER DATA**

After the discovery of COX-2, studies of the effects of NSAIDs on cancer were focused on their relationship with COX-2 induction. In 1994, Eberhart et al. (43) reported that, of 14 human colorectal carcinoma samples, 12 (86%) had marked COX-2 induction. In 1994, Eberhart et al. (43) reported that, of 14 human colorectal carcinoma samples, 12 (86%) had marked COX-2 induction. In 1994, Eberhart et al. (43) reported that, of 14 human colorectal carcinoma samples, 12 (86%) had marked COX-2 induction.

<table>
<thead>
<tr>
<th>Authors (study area)</th>
<th>Year of study</th>
<th>No. of patients studied*</th>
<th>NSAID used</th>
<th>RR [95% CI]† (comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kune et al. (126) (Australia)</td>
<td>1988</td>
<td>715</td>
<td>Aspirin</td>
<td>0.57 [0.41–0.79]</td>
</tr>
<tr>
<td>Rosenberg et al. (127) (Boston, New York City, Philadelphia, and Baltimore)</td>
<td>1991</td>
<td>1326</td>
<td>Aspirin</td>
<td>0.5 [0.4–0.8]</td>
</tr>
<tr>
<td>Suh et al. (128) (Buffalo, NY)</td>
<td>1993</td>
<td>830</td>
<td>Aspirin</td>
<td>0.44/0.83 (dose dependent) [0.18–1.10]/[0.43–1.61]</td>
</tr>
<tr>
<td>Logan et al. (35) (Nottingham, U.K.)</td>
<td>1993</td>
<td>40</td>
<td>Aspirin or other NSAIDs‡</td>
<td>0.49/0.66 (−/+ occult blood tests) [0.3–0.8]/[0.4–0.8]</td>
</tr>
<tr>
<td>Peleg et al. (129) (Atlanta, GA)</td>
<td>1994</td>
<td>97</td>
<td>Aspirin or other NSAIDs‡</td>
<td>0.52–0.08 (dose dependent)</td>
</tr>
<tr>
<td>Peleg et al. (130) (Atlanta, GA)</td>
<td>1996</td>
<td>206 (93 carcinomas + 113 adenomas)</td>
<td>Aspirin or other NSAIDs‡</td>
<td>0.34–0.77 (4-y study)</td>
</tr>
<tr>
<td>Muscat et al. (131) (New York City area)</td>
<td>1994</td>
<td>511</td>
<td>NSAIDs‡</td>
<td>0.31/0.59 (dose dependent) [0.11–0.84]/[0.23–1.48]</td>
</tr>
<tr>
<td>Martinez et al. (132) (Houston, TX)</td>
<td>1995</td>
<td>157</td>
<td>Aspirin and/or NSAIDs</td>
<td>0.64 (males) [0.42–0.97]</td>
</tr>
</tbody>
</table>

**Retrospective studies**

<table>
<thead>
<tr>
<th>Authors (study area)</th>
<th>Year of study</th>
<th>No. of patients studied*</th>
<th>NSAID used</th>
<th>RR [95% CI]† (comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paganini-Hill et al. (36)</td>
<td>1989</td>
<td>68</td>
<td>Aspirin</td>
<td>0.95–1.67</td>
</tr>
<tr>
<td>Paganini-Hill et al. (133) (California retirement community)</td>
<td>1991</td>
<td>79</td>
<td>Aspirin</td>
<td>0.79–1.46 (no reducing effects)</td>
</tr>
<tr>
<td>Thun et al. (134) (U.S.; 50 states, DC, and Puerto Rico)</td>
<td>1991</td>
<td>726</td>
<td>Aspirin‡</td>
<td>0.77–0.60 (males)</td>
</tr>
<tr>
<td>Gann et al. (37) (U.S. physicians)</td>
<td>1993</td>
<td>63 (of 11,037)</td>
<td>Aspirin‡</td>
<td>0.73–0.58 (females) (dose dependent) 1.15 for carcinoma</td>
</tr>
<tr>
<td>Schreinemachers and Everson (135) (U.S.; National Health and Nutrition Examination Survey I)</td>
<td>1994</td>
<td>10</td>
<td>Aspirin</td>
<td>0.86 for in situ carcinoma and polyps</td>
</tr>
<tr>
<td>Giovannucci et al. (136) (U.S. health professionals)</td>
<td>1994</td>
<td>67 (1-y use)</td>
<td>Aspirin</td>
<td>0.35 (males &lt;65 y old) (lung, 0.68; breast, 0.70)</td>
</tr>
<tr>
<td>Giovannucci et al. (137) (U.S. female registered nurses)</td>
<td>1995</td>
<td>149 (1-y use)</td>
<td>Aspirin</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Prospective studies**

<table>
<thead>
<tr>
<th>Authors (study area)</th>
<th>Year of study</th>
<th>No. of patients studied*</th>
<th>NSAID used</th>
<th>RR [95% CI]† (comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paganini-Hill et al. (36)</td>
<td>1989</td>
<td>68</td>
<td>Aspirin</td>
<td>0.95–1.67</td>
</tr>
<tr>
<td>Paganini-Hill et al. (133) (California retirement community)</td>
<td>1991</td>
<td>79</td>
<td>Aspirin</td>
<td>0.79–1.46 (no reducing effects)</td>
</tr>
</tbody>
</table>

*Excluding the control group patient numbers.
†RR = relative risk; CI = confidence interval; values shown are adjusted for various factors or multivariate estimates.
‡Acetaminophen did not show any significant effects.

<table>
<thead>
<tr>
<th>Year of study</th>
<th>No. of patients studied*</th>
<th>NSAID used</th>
<th>RR [95% CI]† (comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>1326</td>
<td>Aspirin‡</td>
<td>0.77–0.60 (males)</td>
</tr>
<tr>
<td>1991</td>
<td>79</td>
<td>Aspirin</td>
<td>0.34–0.77 (4-y study)</td>
</tr>
<tr>
<td>1994</td>
<td>206 (93 carcinomas + 113 adenomas)</td>
<td>Aspirin or other NSAIDs‡</td>
<td>0.31/0.59 (dose dependent) [0.11–0.84]/[0.23–1.48]</td>
</tr>
<tr>
<td>1995</td>
<td>157</td>
<td>Aspirin and/or NSAIDs</td>
<td>0.36 (once a day or more)</td>
</tr>
</tbody>
</table>

4.77 (weekly)
expression of the COX-1 polypeptide was weak in both normal and cancer specimens. Likewise, Kutchera et al. (46) found by in situ hybridization that the neoplastic colonocytes had increased expression of COX-2. In addition, five colon cancer cell lines were shown to express high levels of COX-2 mRNA. By transfection experiments with the 5’ regulatory sequence of the COX-2 gene ligated to a luciferase reporter, the researchers found that colon cancer cell line HCT-116 constitutively expressed COX-2, whereas normal control cell lines transcribed the reporter only in response to an exogenous agonist.

In 1995, several groups reported that sulindac and other NSAIDs induce apoptosis in colon cancer cells. By immunohistochemistry, Pasricha et al. (47) studied colonic biopsy samples from 22 FAP patients who were enrolled in a sulindac trial. The subdiploid apoptotic fraction was significantly increased to 31%, compared with 10% in the controls, 3 months after treatment with sulindac. Likewise, Bedi et al. (48) showed that eight FAP and 10 sporadic adenomas exhibited reduced apoptotic fractions in TdT (terminal deoxynucleotidyltransferase)‐mediated deoxyuridine triphosphate‐digoxigenin nick‐end labeling (TUNEL) and DNA fragmentation assays compared with eight normal colonic epithelial samples. In 11 colorectal carcinoma samples, the reduction of apoptosis was most dramatic, with an abnormal increment in the G2 fraction.

Using HT-29 human colon adenocarcinoma cells in culture, Shiff et al. (49) showed that sulindac and its metabolite sulindac sulfide reduced the cell proliferation rate, changed the cells’ morphology, and induced apoptosis in these cells. Because sulindac is a prodrug, it is metabolized to a pharmacologically active sulfide derivative that inhibits prostanooid synthesis (50). Some studies, however, have shown that a sulfone derivative of sulindac, which essentially lacks prostaglandin synthesis inhibitory activity (50), also inhibits chemical carcinogenesis, suggesting an additional mechanism of antineoplastic activity by sulindac and its metabolites (51). Piazza et al. (52) found that both sulindac sulfide and sulfone significantly reduced the number of HT-29 cells and of a variety of other tumor cell lines, as well as the number of normal epithelial cells and fibroblasts. It is interesting that both sulindac sulfide and sulfone induced apoptosis in HT-29 cells in a time- and dose-dependent manner. Regarding the apoptosis caused by sulindac and its metabolites in tumor cells, it is worth noting that several other NSAIDs, but not sulindac, cause apoptosis in v-src-transformed chicken embryo fibroblasts. At the same time, NSAIDs induce COX-1 and COX-2 mRNA. However, the induced COX-2 transcript is in a partially spliced and nonfunctional form (53). Lu et al. (53) further showed that expression of bcl-2 is very low in these cells and is not affected by NSAID treatment. In contrast, expression of p20, a protein that may protect against apoptosis when fibroblasts enter the G0 phase, was strongly repressed, as shown by northern blot analysis.

Tsujii and DuBois (54) introduced a rat COX-2 complementary DNA (cDNA) driven by the cytomegalovirus promoter into a nontransformed rat intestinal epithelial (RIE) cell line and established clones that express COX-2 continuously (RIE-S). They also constructed control cell lines in which the cDNA was placed in the antisense orientation (RIE-AS). The RIE-S cells expressed elevated COX-2 protein levels and exhibited increased adhesion to extracellular matrix proteins. The RIE-S cells were resistant to butyrate-induced apoptosis, had elevated BCL2 protein expression, and had reduced levels of the type II receptor for transforming growth factor-β (TGF-β). Such phenotypic changes were reversed by sulindac sulfide. These data, considered together, suggest that overexpression of COX-2 in intestinal epithelial cells may enhance their tumorigenic potential.

Recently, Samaha et al. (55) studied the effects of several potential chemopreventive agents on apoptosis in azoxymethane-induced colon tumors in male F344 rats. They found that sulindac, curcumin, and phenylethyl-3-methylcaffeate significantly increased the apoptotic index (percentage of apoptosis) as compared with the control. Ballif et al. (56) reported that an autoimmunity- and apoptosis-associated nucleobindin interacts with both COX-1 and COX-2. It remains to be investigated whether nucleobindin is involved in the inhibition of apoptosis by COX-2.

**COX-2 and Polyposis: Studies With Apc Knockout Mice as a Model for FAP**

Molecular genetic studies of FAP kindreds led to the discovery of the APC gene on human chromosome 5q21 (57–60). Mutations in APC appear to be responsible for not only FAP but also many sporadic cancers of the colorectal axis, stomach, and esophagus (61–63). While most FAP cases have mutations in the upstream half of exon 15 (64), mutations near the 5’ end of the coding region cause an attenuated form of the disease with relatively few colonic polyps (65). Another form of FAP, which is associated with congenital hypertrophy of the retinal pigment epithelium, contains mutations downstream of exon 9 (66). APC consists of 15 coding exons and several 5’ noncoding exons, with various combinations of which generate many isoforms by alternative splicing (59,67,68). The gene encodes a huge protein, about 2840 amino acids in length (57,60). The protein contains regions that may form an α-helical, coiled-coil structure; a subdomain of the first 55 amino acids forms a stable, parallel helical dimer (69). Antibody studies showed that the wild-type, but not mutant, Apc protein is associated with the microtubule cytoskeleton (70,71). The predicted structure of Apc, its localization, and its interaction with β-catenin (72,73) suggested that it is involved in cell adhesion. In fact, studies have demonstrated that Apc is localized to plasma membrane sites involved in active cell migration (74) and in the nucleus as well (75). At the same time, β-catenin interacts with the hTcf-4 and Lef transcription factors. In fact, hTcf-4 transactivates transcription only when associated with β-catenin (76,77).

A dominant mouse mutation, Min (multiple intestinal neoplasia), which was generated by chemical mutagenesis and causes polyposis in the digestive tract, has been located in Apc, the mouse homologue of the human APC gene. It causes truncation of the gene product at codon 850 and multiple polyps in the intestinal tract (78,79). Boolbol et al. (80) reported that both the levels of COX-2 protein and PGE2 production were elevated in the Min mouse intestines, even in the regions where no polyps developed. COX-2 or PGE2 was not elevated in the intestines of the wild-type littermates. Such increases in COX-2 protein and PGE2 in Min intestines were reversed when the
mice were given sulindac in their drinking water, and the polyp number was reduced to 0.1 tumor per mouse compared with 11.9 tumors per mouse in the untreated Min mice. It is interesting that Min mice showed a 27%–47% decrease in enterocyte apoptosis, which was reversed by the sulindac treatment (80).

To investigate the molecular mechanism of polyp formation as a precursor to carcinogenesis in the digestive tract, we earlier constructed gene knockout mice carrying a mutant Apc gene encoding a product truncated at codon 716 (Apc(D716)) (81). Whereas the homozygous mutant mice die in utero before day 8 of gestation, the heterozygotes are viable and develop multiple polyps throughout the intestinal tract, mostly in the small intestine. The earliest polyps arose multifocally during the 3rd week after birth, and new polyps continued to appear thereafter. Surprisingly, every nascent polyp consisted of a microadenoma covered with a layer of the normal villous epithelium. These microadenomas originated from single crypts that formed abnormal outpockets in the inner (lacteal) side of the neighboring villi. We carefully dissected such microadenomas from nascent polyps by peeling off the normal epithelium and determined their genotype by polymerase chain reaction: All microadenomas had already lost the wild-type allele, whereas the mutant allele remained unchanged. These results indicate that loss of heterozygosity (LOH), followed by formation of intravillous microadenomas, is responsible for the microadenoma initiation in Apc(D716) intestinal mucosa (81). This mutant mouse strain provided a useful model system for investigation of various carcinogens and for evaluation of anticancer and chemopreventive agents. In fact, we demonstrated that the heterocyclic amines that are generated in overcooked meat stimulate the growth of the intestinal polyps, whereas feeding the Apc(D716) mice docosahexaenoic acid substantially reduces the number of polyps (82,83).

To examine the expression of COX-1 and COX-2 in the Apc(D716) mice, we first performed immunoblot analyses of polyp proteins by using specific antibodies against COX-1 and COX-2, respectively. The normal intestinal epithelium—as well as the polyps of various sizes—expressed COX-1 protein at similar levels, both in the colon and in the small intestine. In contrast, the normal epithelium of neither the small intestine nor the colon contained any detectable COX-2 protein. However, polyps as small as 2 mm in diameter from either the colon or the small intestine contained substantial levels of COX-2 protein. The results indicate that COX-2 is induced in the polyp tissues at a very early stage of development, long before their malignant transformation (84).

To determine the effect of the absence of COX-2 on Apc(D716) polyp formation, we (84) introduced a knockout mutation of the COX-2 gene (Ptgs2) (40) into the Apc(D716) knockout mouse by successive crosses and constructed compound mutant mice that carried Apc(D716) (+/-) Ptgs2 (+/-) and Apc(D716) (+/-) Ptgs2 (–/-) mutations, respectively. The Apc(D716) (+/-) Ptgs2 (+/+ litters were used as positive controls. When the intestinal polyps were scored at the same age, the polyp numbers in the Apc(D716) (+/-) Ptgs2 (+/-) and Apc(D716) (+/-) Ptgs2 (–/-) mice were reduced to 34% and 14% of the control, respectively (Fig. 1, A). Moreover, the size of the polyps in these mice was statistically significantly smaller than in the controls (Fig. 1, B). To our knowledge, these results are the first direct genetic evidence that COX-2 plays a key role in polyp formation, and they suggest that COX-2 inactivation suppresses polyp growth rather than polyp initiation (84). This is in clear contrast with dietary effects on Apc(D716) (+/-) polyps. We (85) fed Apc(D716) (+/-) mice either a low-fat and high-fiber diet (a low-risk diet) or a high-fat and low-fiber diet (a high-risk diet) for 7 weeks. Although the mice fed a high-risk diet developed polyps in statistically significantly higher numbers than those fed a low-risk diet, both in the small intestine and in the colon, there was essentially no difference in the polyp size distribution between the two groups. It is likely that a high-risk diet increases the frequency of the initial event, i.e., LOH of the Apc gene (85).
To determine whether we can mimic the Ptgs2 knockout mutation by administering pharmaceutical agents to the Apc\(^{−/−}\) mice, we next tested the effects of a novel COX-2 selective inhibitor, MF tricyclic, and a nonselective COX inhibitor, sulindac (84). MF tricyclic is a research compound (Fig. 2, A) that shows more than 100-fold selectivity for COX-2 over COX-1 (i.e., its COX-2/COX-1 IC\(_{50}\) ratio) when compared with that of sulindac (Fig. 2, B). (IC\(_{50}\) = concentration of the compound that causes half-maximal [50%] inhibition of the enzyme.) When mice were fed MF tricyclic at 14 and 3.5 mg/kg per day, the drug reduced polyp numbers by 62% and 50% of that seen in the control, respectively, compared with only a 26% reduction in the polyp number by sulindac at 12 mg/kg per day (Fig. 2, C).

It is interesting that suppression of COX-2 activity, either by introduction of the knockout mutation or by the COX-2 selective inhibitor MF tricyclic, had a profound effect on the polyp morphology as well. Well-developed polyps in Apc\(^{−/−}\) (+/−) Ptgs2\(^{−/−}\) mouse intestines appeared to be recessed from the surface of the surrounding villi. This was primarily due to the presence of fewer stromal (or interstitial) cells compared with Apc\(^{−/−}\) mouse intestines appeared to be recessed from the surface within the intestinal mucosa. From analysis of human colorectal cancer tissues by use of in situ hybridization, Kutcher et al. (46) found strong COX-2 mRNA signals in the tumor cell area rather than in the stromal area. In a histochemical analysis, Sano et al. (45) reported staining of COX-2 in both cancer epithelium and stromal cells such as inflammatory cells, vascular endothelium, and fibroblasts. The discrepancy between these observations and ours may be explained in two ways: One depends on the stage in the tumor’s development, and the other relies on technical details of the immunohistochemistry. We looked at an early stage of polyp development (84), whereas Williams et al. (87) looked at much more advanced tumors. In advanced tumors, many secondary reactions take place, such as the proliferation of stromal cells and tissue remodeling, showing a histologic picture very different from that of early tumors. Although several COX-2-specific antibodies have been described, and some are commercially available, many of them show cross-reacting bands upon immunoblot analysis at a high sensitivity. It is also worth noting that the major prostaglandin found in colorectal cancer tissues is PGE\(_2\) (88,89). In contrast, when a rat intestinal epithelium (RIE-1) cell line is stimulated by TGF-α or tissue plasminogen activator, the major prostaglandin secreted into the medium is 6KPGF\(_{1α}\), the nonenzymatic hydrolysis product of prostacyclin (PGL\(_{1}\)) (90).

These results have several implications and present important questions for future research, as pointed out by Prescott and White (91).

**Questions for the laboratory researcher:** 1) How does COX-2 expression become dysregulated after loss of APC function? 2) Is the dysregulation transcriptional and, if so, through which factors? 3) Is COX-2 expression alone sufficient to cause colon neoplasia? 4) What are the important metabolites of the COX-2 product and what signaling pathways do they influence? 5) Which cellular responses (e.g., loss of apoptosis) lead to tumors?

**Questions for the clinician:** 1) Will specific inhibitors of COX-2 be more effective than nonselective NSAIDs? 2) Will inhibition of COX-2 be as effective in patients with sporadic polyps and HNPCC as it is in patients with FAP? 3) What accounts for the residual cases of neoplasia during treatment with NSAIDs—is it a rare event, that occurs only in some early polyps, or will all of the polyps eventually escape the inhibitory
effect? 4) How should chemoprevention with COX inhibitors be integrated into current surveillance and intervention protocols? (91)

Answers to some of these questions are already in hand. Using a human colon cancer cell line, HCA-7, cultured on Transwell filters, Coffey et al. (92) succeeded in establishing a polarized cell population. When the cells were stimulated by TGF-α from the basolateral compartment, where the epidermal growth factor receptor (EGFR) resides, a marked secretion of prostaglandins was observed in the basolateral but not in the apical medium, followed by mitogenesis. Two specific COX-2 inhibitors, SC-58125 and NS-398, were found to attenuate COX-2 induction and subsequent mitogenesis. These data indicate that activation of EGFR stimulates COX-2 biosynthesis, vectorial release of prostaglandins, and mitogenesis in polarized HCA-7 cells (92). In addition to the HCA-7 line, which express high levels of COX-2 protein, Sheng et al. (93) studied the HCT-116 cell line, which lacks COX-2 expression. Treatment of nude mice implanted with HCA-7 cells with a selective COX-2 inhibitor, SC-58125, reduced tumor formation by 85%–90%. SC-58125 also inhibited colony formation of HCA-7 cells in culture. Conversely, SC-58125 had no effect on HCT-116 implants in nude mice or on HCT-116 colony formation in culture.

The effects of several NSAIDs and another COX-2 inhibitor were evaluated on carcinogen-induced colonic aberrant crypt foci (ACF) in rats. Reddy et al. (94) assessed the chemopreventive properties of SC-58635, a COX-2 inhibitor, and of sulindac against azoxymethane-induced colonic ACF in male F344 rats. Administration of 1500 ppm SC-58635 in the diet induced total ACF induction and crypt multiplicity by 40%–49%, whereas administration of 330 ppm sulindac in the diet induced ACF multiplicity by about 35%. Barnes et al. (95) tested various compounds in DMH-treated male Sprague-Dawley rats. Only aspirin, but not sodium salicylate, indomethacin, or nabumetone, reversibly suppressed colonic ACF.

Several reports described the results of NSAID trials on sporadic colonic polyps. Hixson et al. (96) studied five sulindac-treated (400 mg/day) and two piroxicam-treated (20 mg/day) patients who completed 6 months of therapy. With the exception of two patients who showed partial response (one treated with sulindac and the other treated with piroxicam), all the patients remained unchanged. Ladenheim et al. (97,98) tested 22 patients with sulindac (300 mg/day) for 4 months and an additional group of 22 patients with placebo. Essentially no differences were found between the two groups. In contrast, Matsuhashi et al. (99) recently reported the results of a study in which 20 patients were treated with sulindac (300 mg/day) for 4 months. In their study, 13 of 20 polyps shrank or disappeared. However, to evaluate the effects of NSAIDs on sporadic polyps, it would be more meaningful to determine whether these polyps have elevated COX-2 and, if they do, to challenge them with COX-2 selective inhibitors.

### NSAIDs and Other Cancers

Studies on animal models showed that the NSAIDs indomethacin, sulindac, ketoprofene, phenylbutazone, and aspirin suppress malignant tumors in experimental animals. As summarized in Table 4, these include both transplanted tumors and autochthonous tumors caused by chemical carcinogens and avian retrovirus. It is interesting that NSAIDs suppress not only cancers of epithelial origin but also tumors of mesenchymal origin, such as sarcomas and mast cell tumors.

After the discovery of COX-2, several papers were published on the role of COX-2 in cancers other than colorectal cancer. Subbaramaiah et al. (100) studied the expression of COX-2 in mouse mammary epithelial cells transformed by either src or rauscher retrovirus. It is interesting that NSAIDs suppress not only cancers of epithelial origin but also tumors of mesenchymal origin, such as sarcomas and mast cell tumors.

![Table 4. Other animal cancers that nonsteroidal anti-inflammatory drugs (NSAIDs) were effective in suppressing](https://academic.oup.com/jnci/article-abstract/90/21/1609/2519718)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cancer</th>
<th>Carcinogen*</th>
<th>NSAID</th>
<th>References and comments†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Fibrosarcoma (transplanted)</td>
<td>MCT</td>
<td>Indomethacin</td>
<td>Tashjian et al., 1974 (17) (PGE₂ and carboxynicinomas increased)</td>
</tr>
<tr>
<td></td>
<td>Fibrosarcoma (transplanted)</td>
<td>MCT</td>
<td>Indomethacin and aspirin</td>
<td>Plescia et al., 1975 (15)</td>
</tr>
<tr>
<td></td>
<td>Mast cell tumor (transplanted)</td>
<td>MCT</td>
<td>Aspirin and indomethacin</td>
<td>Lynch et al., 1978 (138)</td>
</tr>
<tr>
<td></td>
<td>Lewis lung carcinoma (transplanted)</td>
<td>Spontaneous</td>
<td>Indomethacin</td>
<td>Hial et al., 1976 (16)</td>
</tr>
<tr>
<td></td>
<td>Esophageal tumor</td>
<td>DENA</td>
<td>Indomethacin</td>
<td>Young and Knes, 1984 (139)</td>
</tr>
<tr>
<td></td>
<td>Bladder carcinoma</td>
<td>BHBN</td>
<td>Sulindac and ketoprofene</td>
<td>Rubio, 1984 (140)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rao et al., 1996 (141) (aspirin, n/e)</td>
</tr>
<tr>
<td></td>
<td>Sarcoma</td>
<td>MSV</td>
<td>Indomethacin</td>
<td>Straußer and Humes, 1975 (142)</td>
</tr>
<tr>
<td></td>
<td>Bladder carcinoma</td>
<td>FANFT</td>
<td>Aspirin</td>
<td>Murasaki et al., 1984 (143) (forestomach carcinomas increased)</td>
</tr>
<tr>
<td></td>
<td>Mammary tumor</td>
<td>DMBA</td>
<td>Indomethacin</td>
<td>Carter et al., 1989 (144) (carprofen, n/e)</td>
</tr>
<tr>
<td></td>
<td>Bladder carcinoma</td>
<td>BHBN</td>
<td>Aspirin</td>
<td>Klan et al., 1993 (145)</td>
</tr>
<tr>
<td>Hamster</td>
<td>Pancreatic carcinoma</td>
<td>BOP</td>
<td>Indomethacin and phenylbutazone</td>
<td>Takashiki et al., 1990 (146) (aspirin, n/s)</td>
</tr>
</tbody>
</table>

* MCT = 3-methylcholanthrene; DENA = N-nitrosodietethylamine or diethylnitrosamine; BHBN = N-butyl-N-(4-hydroxybutyl)nitrosamine or OH-BBN; MSV = Moloney sarcoma virus; FANFT = N-(4-(5-nitro-2-furyl)-2-thiazolyl)-formamide; DMBA = 7,12-dimethylbenz[a]anthracene; and BOP = N-nitrosobis (2-oxopropyl)amine.

† PGE₂ = prostaglandin E₂; n/e = no effect; PGs = prostaglandins; n/s = not significant.
human prostate cancer cell lines PC-3 and LNCaP, as well as human breast and colorectal cancer cell lines, with dimethylprostaglandin E2 in culture. This compound increased the COX-2 mRNA level and the cell growth rate, while the NSAID flurbiprofen (5 mM) inhibited the up-regulation (increased expression) of COX-2 mRNA and the stimulation of PC-3 cell growth that occurs in the presence of dimethylprostaglandin E2.

Although PGE2 has tumor and cell growth-promoting activity, its dehydrogenation products PGA2 and PGJ2 have been shown by Fukushima et al. (102–105) to inhibit cell growth in vitro and to exhibit antitumor activity in vivo. Gorospe et al. (106) showed in the human breast carcinoma cell line MCF-7 that PGA2 treatment causes arrest in phase G0 of the cell cycle and a dramatic decrease in the levels of cell cycle-related proteins cyclin D1 and cyclin-dependent kinase 4, together with an increase in p21 gene and protein expression, independent of p53 status. In the human colorectal carcinoma cell line RKO, PGA2 treatment fails to induce growth arrest; instead, it results in substantial cell death. These effects are associated with a lack of p21 induction and with enhanced cyclin-dependent kinase 2 activity (107).

**CONCLUSION**

Genetic and pharmacologic evidence has established that COX-2 is induced in the polyps of Apc<sup>min</sup> and Min mice, two mouse models of human FAP. Selective (or specific) COX-2 inhibitors are much more efficient in suppressing polyposis in these mice or in suppressing ACF induced in rats than are traditional NSAIDs; furthermore, these compounds have the advantage of not causing gastrointestinal side effects. Many additional animal experiments and clinical trials using COX-2 selective inhibitors will be undertaken in coming years to establish the role of these compounds in chemotherapy for polyposis and for various other cancers, as well as in cancer chemoprevention. Before rushing these compounds into clinical trials, however, it would be important for us to determine whether COX-2 is induced and plays a key role in the cancer and/or precancerous condition that is the target of a particular trial. Once this association is established, we can reasonably expect that treatments with COX-2 selective inhibitors will bring us promising chemotherapeutic effects.

**REFERENCES**

(34) LOGAN RF, Littel J, Hawtin PG, Hardcastle JD. Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case–con-


NOTES

1 Prostanoids is a more accurate term than prostaglandins when all physiologically active metabolites of prostaglandin H2 are indicated; i.e., prostaglandins A2, D2, E2, F2α, I2 (prostacyclin), J2, and thromboxane A2.

Editor’s note: Part I of this review, which appears in the Vol. 90, No. 20, October 21, 1998, issue of the Journal, focuses on the discovery of the cyclooxygenases (COXs); their biochemical, molecular, and structural properties; and their discovery of isozyme-specific inhibitors of COX activity.

Dedicated to Sir Professor John Vane and Professor Osamu Hayaishi whose works inspired me into this fascinating field of research.

I am grateful to the following colleagues who collaborated with me on some of the work referred in this review: Masanobu Oshima, Hiroko Oshima, Kyoko Kitagawa, Masahiko Kobayashi, Susumu Nishimura, Chitoshi Itakura, Masahiro Tsutsumi, Mami Takahashi, Keiji Wakabayashi, Minako Nagao, Takashi Sugimura, Kazunaga Yazawa, and Kyoji Hikoi (Japan); Joseph E. Dinchuk, James M. Trzaskos, Narayan Shivapurkar, and Oliver Alabaster (United States); and Stacia L. Kargman, Bruno Hancock, Elizabeth Kwong, and Jilly F. Evans (Canada). I also thank Tetsuo Nagano and Yoshinori Sato for fruitful discussions.

Manuscript received October 24, 1997; revised July 8, 1998; accepted September 2, 1998.