Inhibition of Cyclooxygenase: A Novel Approach to Cancer Prevention (44170)

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Abstract. **An expanding body of evidence indicates that downregulation of the cyclooxygenases (Cox-1 and Cox-2) will be an important strategy for preventing cancer because cyclooxygenases catalyze the formation of prostaglandins (PGs), and PGs have multiple effects that favor tumorigenesis. PGs also are more abundant in cancers than in the normal tissues from which cancers arise. Overexpression of Cox-2 in epithelial cells inhibits apoptosis and increases the invasiveness of tumor cells; inhibitors of Cox (e.g., NSAIDS) are chemopreventive; and tumorigenesis is inhibited in Cox-2 knockout mice. We focus in this review on strategies to selectively inhibit and downregulate the Cox-2 isoform. This is important because simultaneous inhibition of Cox-1 (constitutively expressed) and Cox-2 (inducible isoform), which is achieved with classical NSAIDs, interferes with the housekeeping functions of Cox-1 and thereby causes serious side effects, such as peptic ulcer disease. Simultaneous inhibition of Cox-1 and Cox-2 hence is not a realistic approach for chemoprevention in individuals at low to moderate risk for cancer. On the other hand, it appears possible to avoid many NSAID-dependent side effects by selective inhibition of Cox-2, which is also the isoform that is upregulated in benign and malignant tumors. Through understanding the biochemistry of these enzymes and the regulation of Cox-1 and Cox-2 gene expression, we review how Cox-2 can be regulated selectively as a target for chemopreventive therapy. We also discuss the potential importance and advantages of a multifaceted approach to diminishing the function of Cox-2 (i.e., combining inhibitors of enzyme function with inhibitors of gene expression).**

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Prostaglandins (PGs) appear to be important in the pathogenesis of cancer because they affect mitogenesis, cellular adhesion, immune surveillance, and apoptosis. Moreover, cancers (e.g., cancer of the head and neck, breast, lung, and colon) form more PGs than the normal tissues from which they arise (1–9). The proposal that PGs contribute to carcinogenesis is supported further by compelling evidence that inhibitors of cyclooxygenase (Cox) activity (and thereby of PG formation) protect against colon, mammary, esophageal, lung, and oral cancer in animals and humans (10–25).

The increased amounts of PGs in tumors reflect enhanced synthesis, which occurs by Cox-catalyzed metabolism of arachidonic acid. PGs are synthesized from arachidonic acid by two different isoforms of Cox, designated Cox-1 and Cox-2. Cox-1 is a constitutive isoform present in most tissues; it mediates the synthesis of PGs required for normal physiological functions. Cox-2 is not detectable in most normal tissues, but it is induced by cytokines, growth factors, oncogenes, serum, and tumor promoters (26–31). Increased production of PGs in transformed cells and tumors is associated with upregulation of Cox-2 (32–36). A possible cause-and-effect connection between the activity of Cox-2 and production of PGs, on the one hand, and the induction of tumors, on the other, was highlighted by recent studies of tumor formation in the gut. A null mutation for Cox-2 caused a marked reduction in the number and size of intestinal polyps in a murine model of familial adenomatous polyposis, i.e., $APC^{\Delta 716}$ knockout mice (37). This result suggests, therefore, that targeted inhibition of Cox-2 could be an effective approach for preventing cancer. This review focuses on our current understanding of the relationship between the activity of Cox and carcinogenesis, and on

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chemopreventive strategies to decrease Cox activity, including our efforts to inhibit the expression of Cox-2.

Metabolism of Arachidonic Acid

Arachidonic acid is an essential, 20-carbon, polyunsaturated fatty acid consumed in the diet or derived from elongation and desaturation of ingested linoleic acid (38, 39). Arachidonate is relatively abundant in cells but occurs almost exclusively at the 2 position of membrane phospholipids. As shown in Figure 1, the first step in synthesis of PGs is the release of arachidonate by hydrolysis of phospholipids, in reactions catalyzed by phospholipases. This step is essential because only free arachidonate is a substrate for further metabolism catalyzed by three different types of oxygenases: cytochrome P-450, lipoxygenases, and cyclooxygenases. Therefore, the availability of free arachidonate is frequently a rate-limiting determinant of PG synthesis.

Cytochrome P-450 converts arachidonic acid to epoxyarachidonic acids (41); lipoxygenases introduce one molecule of O_2 into arachidonate to produce a series of isomeric, hydroperoxyacid products including leukotrienes and lipoxins (42), and cyclooxygenases oxygenate arachidonic acid to prostaglandin G_2 (PGG_2). This unstable intermediate is then converted by the peroxidase activity of Cox to PGH_2, which is the common precursor for all prostanoids. PGH_2 is further metabolized by different but specific synthases to produce a variety of eicosanoid products (e.g., PGs, thromboxanes, and prostacyclins) (43). By-products of these reactions such as malondialdehyde, which is highly reactive itself, can form adducts with DNA and also may contribute to carcinogenesis (44, 45). We note that because free arachidonate is a substrate for several metabolic pathways and

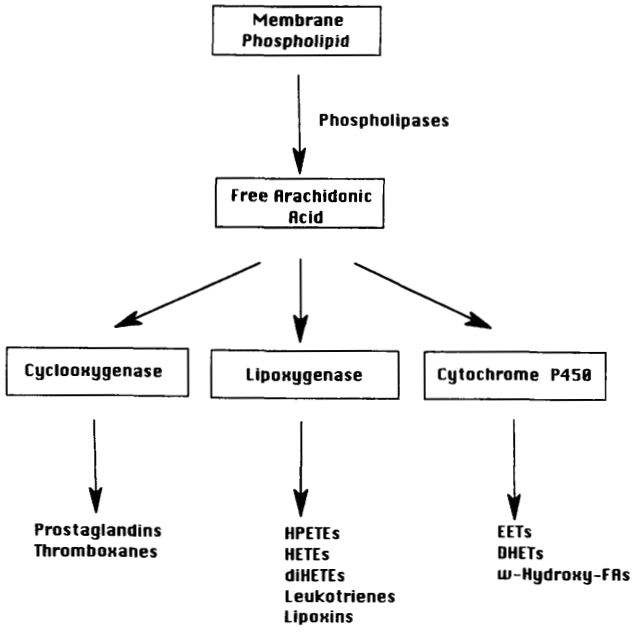

Figure 1. Metabolic pathways for arachidonic acid. (Adapted from Ref. 40.)

in short supply, changes in the activity of one enzyme (e.g., induction of Cox-2) can affect the relative amounts of biologically active products formed by competing metabolic pathways. This means that the biological consequences of changes in Cox activity may depend on changes in the concentrations of metabolites produced by lipoxygenase and P-450 in addition to PGs.

Prostaglandins are synthesized rapidly upon cell stimulation and secreted immediately, to act locally as autacoids or hormones through cell-surface, G protein–linked receptors (46). However, the specific types and amounts of PGs and thromboxanes formed by Cox-catalyzed oxidation of arachidonate depend on the exact composition of different synthases in different types of cells. Additionally, each of the products derived from PGH_2 has its own range of biological activities. PGI_2 and PGE_2, for example, are vasodilatory (47); TXA_2 is a vasoconstrictor and aggregates platelets (48). Prostanoids such as $PGF_{2\alpha}$ and PGE_2 are important in reproduction, ovulation, luteinization, sperm migration, fertilization, implantation, and fetal development. Among many other functions, PGs are important for maintaining normal gastrointestinal and kidney function. Peptic ulcer disease (39, 49) and decreased kidney function (51) are complications of treatment with NSAIDs, presumably because these drugs inhibit synthesis of PGs. In view of the complexity of effects due to eicosanoids and their importance for maintaining normal physiologic function, complete inhibition of PG synthesis is not a rational therapeutic goal. Minimizing side effects secondary to inhibition of PG synthesis hence is a key problem in developing effective chemopreventive measures that are based on inhibition of PG synthesis. Selective inhibition of the synthesis of some but not all PGs is a possibility, however, because of the segregation of synthetic activity between Cox-1 and Cox-2, and important differences in the structures of these isoforms.

The availability of selective inhibitors of Cox-2 is especially important given the multiple lines of evidence suggesting a link between levels of Cox-2 and tumorigenesis. Cox-2 is upregulated in human cancers of the colon (34–36), stomach (52), and breast (53). Increased levels of Cox-2 are also detected in premalignant intestinal tumors in humans (34) and in experimental animals (54). As mentioned, knocking out the Cox-2 gene led to a marked reduction in the number and size of polyps in $Apc^{\Delta 716}$ mice (Fig. 2). Moreover, use of a selective Cox-2 inhibitor suppressed polyp formation in $Apc^{\Delta 716}$ mice by about 60% (Fig. 3). In a separate study, SC-58635, another selective inhibitor of Cox-2, suppressed the formation of aberrant crypt foci in the intestines of azoxymethane-treated rats (55). Cox deficiency also appears to protect against the formation of extraintestinal tumors. Cox-1 and Cox-2 knockout mice developed fewer skin papillomas than controls (56). In combination, these results suggest that inhibition of Cox-2 could be important for preventing the formation of a variety of epithelial tumors. Whether simultaneous inhibition of Cox-1

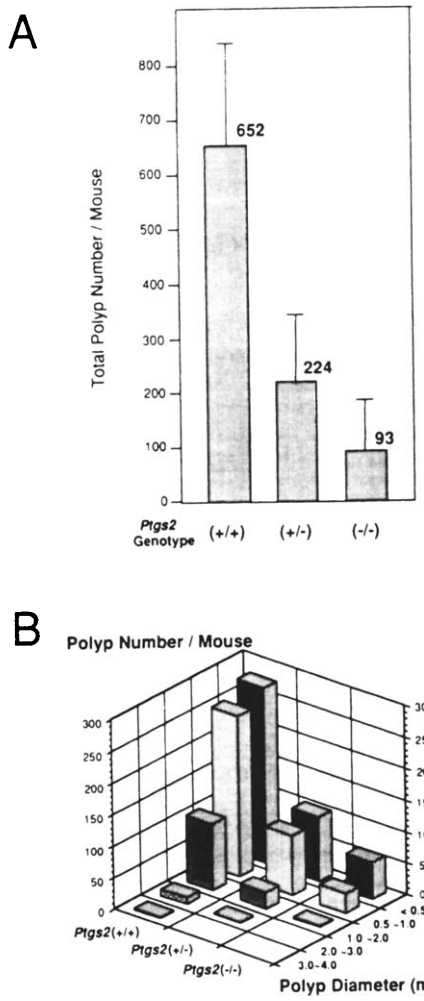

Figure 2. Effects of Ptgs2 (Cox-2) mutations on intestinal polyps in $Apc^{\Delta 716}$(+/–)Ptgs2(+/–) and $Apc^{\Delta 716}$(+/–)Ptgs2(–/–) mice, compared with the $Apc^{\Delta 716}$(+/–)Ptgs2(+/+) controls. (A) The mean number of polyps per mouse is shown, with SD. (B) Size distribution of the intestinal polyps. Polyp sizes were classified according to their diameters in millimeters. Sample, n = 8 randomized mice for each group. (Reprinted with permission from Ref. 37.)

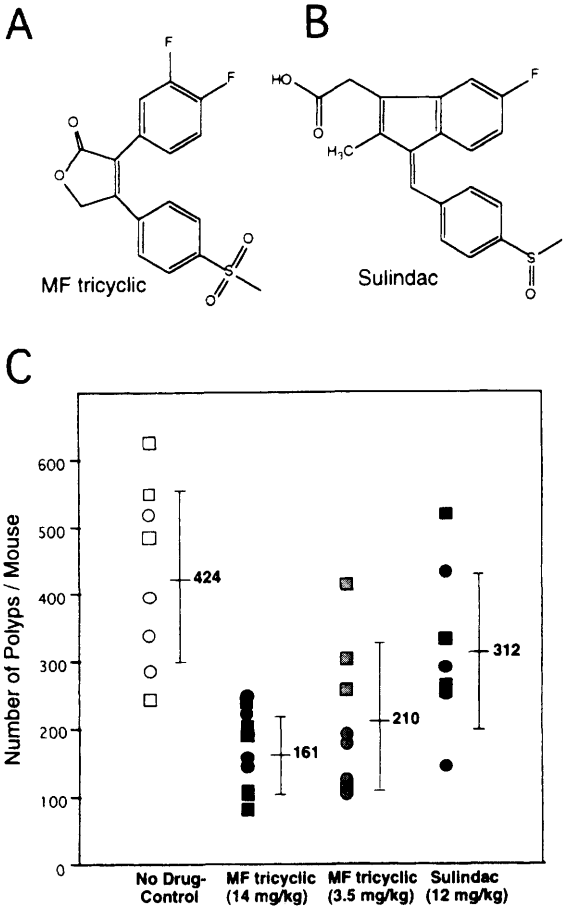

Figure 3. Effect of a novel Cox-2 inhibitor MF tricyclic and sulindac on $Apc^{\Delta 716}$(+/–) mouse intestinal polyps. (A) Structure of MF tricyclic. (B) Structure of sulindac. (C) Number of polyps per mouse scored in $Apc^{\Delta 716}$(+/–) mice fed with the control diet, or diet with MF tricyclic or sulindac. Circles, polyp numbers for individual females; squares, males; Number and vertical bar to the right of each sample group, the mean number and SD, respectively. The drug doses have been calculated from the concentrations of the drugs in the diet and the actual diet intakes. (Reprinted with permission from Ref. 37.)

would result in any additional chemopreventive benefit is an important but unresolved issue.

Cyclooxygenases

The two isoforms of Cox are about 60% homologous within a species. Moreover, all amino acids identified as important for catalysis by Cox-1 are conserved in Cox-2. On the other hand, there are significant differences between the gene and promoter structures of Cox-1 and Cox-2, the stability of their mRNAs, and the intracellular localization of the gene products. Each of these features is important for understanding differences in function. Table I summarizes some of the similarities and differences between the genes for Cox-1 and Cox-2, and between the isozymes themselves.

Complementary DNAs encoding human, mouse, rat, and sheep Cox-1 have been cloned (57–61). The protein sequences are about 90% homologous. The size of the sheep, rat, mouse, and human mRNAs is approximately 2.7 kb. In 1989, Simmons *et al.* identified Cox-2 (62). This Cox isoform was also identified by differential screening of a phorbol ester–stimulated Swiss-3T3 fibroblast cDNA library (26). Cox-2 now has been cloned as an inducible, immediate early response gene from human (63), mouse (26, 64), rat (59, 65), and chicken (66).

Cox-1 is on human chromosome 9 (67), whereas Cox-2 is located on human chromosome 1 (26, 68). The two genes also differ in size. Cox-1 is about 22 kb in length and contains 11 exons and 10 introns; Cox-2 is approximately 8 kb and contains 10 exons and 9 introns (69–71). Although the exons of the two genes are similar in size, the introns of the Cox-2 gene are considerably smaller than those of the Cox-1 gene. The mRNA of Cox-2 is about 4.5 kb long and is unstable compared with Cox-1 mRNA; the instability of Cox-2 mRNA has been attributed to the presence of 17 copies of the Shaw-Kamen sequence (AUUUA) in the 3′-untranslated region of Cox-2 (68, 72). These instability

Table I. A Comparison of Cox-1 and Cox-2

	Cox-1	Cox-2
Regulation	Constitutive	Inducible
Range of expression	Can increase 2 to 4-fold	Can increase 10- to 80-fold
Protein size	Single band of about 72 kDa on SDS-PAGE	Doublet on SDS-PAGE with M_rs of 72 and 74 kDa
C terminus	18–amino acid cassette absent	18–amino acid cassette present
Prosthetic group	Heme	Heme
Gene size	22 kb	8.3 kb
Human chromosome	9	1
mRNA size	2.7 kb	4.5 kb; contains multiple Shaw-Kamen sequences
Localization	Endoplasmic reticulum	Endoplasmic reticulum, nuclear envelope
Effect of glucocorticoids	Little or none	Inhibit expression
Cell and tissue expression	Platelets, stomach, kidney, colon, most tissues	Parts of brain, activated macrophages, synoviocytes during inflammation, malignant epithelial cells, expressed in most cells or tissues after stimulation with cytokines, growth factors or tumor promoters

sequences are present in many immediate-early response genes. The 5′-flanking region of Cox-2 contains a TATA motif; Cox-1 does not contain a classical TATA box, a feature typical for housekeeping genes. The 5′-flanking region of the Cox-2 gene contains numerous *cis*-acting promoter elements, including NF-κB, NF-IL6, and CRE sites (Fig. 4). Experiments with reporter plasmids containing the Cox-2 promoter and 5′-flanking sequence have shown that these elements are important for mediating gene expression (73).

Based on its cDNA sequence, the predicted molecular weight of Cox-1 is 65.5 kDa, excluding the signal peptide. However, Cox-1 migrates as a single band on SDS-PAGE, with an M_r of about 72 kDa. This difference between the predicted and observed values of M_r is a consequence of the post-transcriptional addition of three, N-linked oligosaccharides at Asn67, Asn143, and Asn409 (74). In contrast to Cox-1, Cox-2 usually appears as a doublet on SDS-PAGE with M_rs of 72 and 74 kDa, respectively. The 72-kDa form contains three N-linked oligosaccharides. The 74-kDa species contains in addition a fourth N-linked oligosaccharide, at Asn 580. This site is only partially glycosylated, yielding two populations of Cox-2 molecules that move slightly differently on a gel (74). The carboxyl terminus of Cox-2 contains a unique 18–amino acid region that is absent in Cox-1. Antibodies raised against this unique 18–amino acid carboxyl-terminal peptide region are used to distinguish Cox-2 from Cox-1 (75).

Although the overall structures of Cox-1 and Cox-2 are very similar, small differences between the NSAID-binding sites of Cox-1 and Cox-2 have been exploited in developing selective inhibitors of Cox-2 (76–78). The replacement of isoleucine by valine in the NSAID-binding site of Cox-2, for example, results in an additional pocket off to the side of the central active site channel of Cox-2. Compounds designed to bind in this additional space are potent and selective inhibitors of Cox-2.

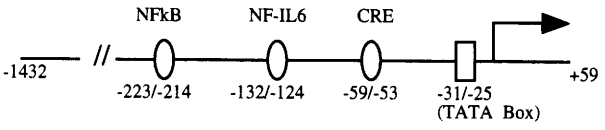

Figure 4. Schematic representation of the human Cox-2 promoter. (Adapted from Ref. (73.)

Cox-1 and Cox-2 are both membrane bound, but have different patterns of subcellular localization. Cox-1 is present primarily in the endoplasmic reticulum (ER). Cox-2 is located in both the ER and the perinuclear envelope (79). Possibly then, PGs produced by the different isozymes may be compartmentalized, with the products of Cox-2 having important effects in the nucleus (e.g., regulating the expression of target genes). Recent data indicate too that Cox-1 and Cox-2 utilize different intracellular pools of arachidonic acid (80), which is likely to have functional consequences.

Gene disruption experiments in mice show that inactivation of Cox-1 leads to a different phenotype versus inactivation of Cox-2. Cox-1 null mice survive well, have no gastric pathology, and are more resistant to indomethacin-induced gastric ulceration than wild-type mice (81). Homozygous Cox-1 mutant mice also have a decreased inflammatory response to arachidonic acid and reduced platelet aggregation (81). In contrast, Cox-2 null mice have normal inflammatory responses to arachidonic acid and normal platelet function. Mice lacking Cox-2 develop nephropathy, cardiac fibrosis, and ovarian defects (82), and are susceptible to peritonitis (83).

Role of Cox in Carcinogenesis

Xenobiotic Metabolism. It is generally accepted that many environmental chemicals are procarcinogens, and that environmental factors are important contributors to the pathogenesis of cancer. Thus, procarcinogens are metabo-

lized in cells to electrophiles that can be proximate carcinogens. For example, the polycyclic aromatic hydrocarbons (PAH) (e.g., benzo[*a*]pyrene B(*a*)P]) present in cigarette smoke and tars are oxidized to produce mutagens, which form covalent adducts with nucleophilic DNA. In liver, these kinds of oxidative reactions are catalyzed principally by cytochrome P-450s; but extrahepatic tissues frequently have low concentrations of these enzymes and other monooxygenases. In this circumstance, significant amounts of PAHs may be co-oxidized by the peroxidase activity of Cox during the metabolism of arachidonic acid (84, 85). The data in Table II indicate that many chemicals are converted to mutagens *via* Cox-mediated metabolism of arachidonic acid (86).

Cox catalyzes the conversion of B[*a*]P–7,8-dihydrodiol to B[*a*]P-diolepoxide (BPDE), which binds to DNA (87, 88). The importance of this reaction is underscored by the recent report that BPDE forms adducts along exons of the *p53* gene that correspond to the *p53* mutational hot spots in human lung cancer (89). In addition, benzidine is oxidized by the peroxidase component of Cox, and activated benzidine can form adducts with proteins and DNA (90). Indeed, the high incidence of urinary-bladder cancer among workers in the dye, chemical, and rubber industries has been attributed to exposure to aromatic amines such as benzidine (91).

The extent of formation of adducts between cellular components and reactive metabolites depends on the balance between the rates of oxidation of the parent compounds and the rates of detoxification of oxidized products *via* conjugation, primarily with glutathione and glucuronic acid. Not surprisingly then, inherited defects in these conjugation reactions predispose to the formation of covalent adducts with DNA (92). It is logical to expect, therefore, that increasing levels of carcinogen-activating enzymes such as Cox-2 will enhance mutagenesis of DNA and favor tumor formation.

Table II. Chemicals Converted to Mutagens during Arachidonic Acid Metabolism in Ames Salmonella Tester Strains

Chemicals
Benzidine and analogs
N-Acetylbenzidine
2,4-Diaminoanisole
2,5-Diaminoanisole
Aromatic amines
2-Aminofluorine
2-Naphthylamine
Heterocyclic aromatic amines
2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)
2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ)
Polycyclic aromatic hydrocarbons
7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP-7,8-diol)
1,2-Dihydro-1,2-dihydroxychrysene (Chrysene-1,2-diol)
3,4-Dihydro-3,4-dihydroxybenzo[*a*]anthracene (BA-3,4-diol)
Cyclopentenyl[*c,d*]pyrene (CPP)

(Reprinted with permission from Ref. 86.)

Separate from the issue of Cox-mediated oxidation of PAHs, chemical carcinogens such as PAHs also stimulate the production of PGs (93) by inducing Cox activity (94). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), for example, enhances the transcription of Cox-2 *via* an aryl hydrocarbon receptor–independent mechanism (95). We have shown too that B[*a*]P can upregulate expression of the Cox-2 gene in human oral epithelial cells (96). These data raise the possibility that B[*a*]P-mediated induction of Cox-2 facilitates its oxidation to BPDE, thereby amplifying the effect of a given dose of B[*a*]P on tumor initiation.

Apoptosis and Invasiveness. Rat intestinal epithelial cells, stably overexpressing Cox-2, have enhanced ability to bind to extracellular matrix proteins and are resistant to undergoing apoptosis when stimulated by butyrate (97). Overexpression of Cox-2 in rat intestinal epithelial cells also is associated with enhanced expression of Bcl-2 protein and decreased expression of both transforming growth factor–β2 receptor (TGFβ2) and E-cadherin (97). TGFβ2 receptors transduce signals that are important for inhibiting the growth of epithelial cells, and E-cadherin is involved in cell-cell adhesion. Each of these changes could enhance the tumorigenic potential of epithelial cells. Treatment with sulindac sulfide, which inhibits Cox-1 and Cox-2, reversed the resistance to apoptosis induced by overexpressing Cox-2 (97). Thus, elevated levels of Cox-2 are associated with resistance to apoptosis, and NSAIDs reverse this effect. Possibly, upregulation of Cox-2 prolongs the survival of abnormal cells, which favors the accumulation of sequential genetic changes and increases the risk of tumorigenesis.

Recently, human colon cancer cells were permanently transfected with a Cox-2 expression vector. These cells acquired increased invasiveness compared with control or parental cells (97). Biochemical changes associated with increased invasiveness included increased RNA levels for the membrane-type of metalloproteinase and activation of metalloproteinase-2. Increased production of PGs and invasiveness both were reversed by treatment with sulindac sulfide (98). These results again support a role for Cox-2 in the pathogenesis of cancer—that is, that constitutive expression of Cox-2 in a cancer cell line leads to phenotypic changes associated with increased metastatic potential.

Inflammation and Immunosuppression. Chronic inflammation is a recognized risk factor for epithelial carcinogenesis (99). Inflammation *per se* increases the synthesis of PGs, at least in part, due to upregulation of Cox-2. The data already reviewed provide a basis for a cause-and-effect link between chronic inflammation and carcinogenesis *via* overexpression of Cox-2, and thereby provide a novel mechanism by which chronic inflammation increases the risk of cancer. Additionally, the growth of various tumors is often associated with immune suppression (100, 101). Colony-stimulating factors released by tumor cells activate monocytes and macrophages to synthesize PGE_2, which inhibits the production of immune regulatory

lymphokines, T- and B-cell proliferation, and the cytotoxic activity of natural killer cells (102–106). PGE_2 also inhibits the production of tumor necrosis factor while inducing the production of IL-10, which has immunosuppressive effects (107, 108). Inhibitors of Cox such as aspirin, sulindac, and indomethacin attenuate tumor-mediated immune suppression (25, 109–111).

NSAIDs and Cancer Prevention

Epidemiological studies have shown that chronic intake of NSAIDs reduces the incidence of colon and breast cancer (14, 16, 17, 20, 21). Synthetic and natural inhibitors of arachidonic acid metabolism protect against mammary, esophageal, oral, and colon cancer in experimental animals (9–13, 18, 19, 22, 23, 112). The NSAID sulindac causes a decrease in the number and size of polyps in Min mice (13) and patients with familial adenomatous polyposis (14). The protective effect of NSAIDs may be explained, at least in part, by the induction of apoptosis (13, 113, 114).

All currently available NSAIDs inhibit both Cox-1 and Cox-2 by competing with arachidonate for binding to the cyclooxygenase active site. NSAID-mediated inhibition of Cox-1 abrogates the crucial housekeeping effects of the products of this constitutively expressed enzyme thereby causing side effects. The use of NSAIDs to prevent cancer has been limited, therefore, by adverse effects such as peptic ulcer disease and renal toxicity (49, 51, 115). The discovery that Cox-2 and Cox-1 have structurally different binding sites for NSAIDs has been important in the development of selective inhibitors for the former enzyme (76–78). Recently, inhibitors of PG synthesis have been developed that are more than 1000 times more selective for Cox-2 than Cox-1 (116, 117).

Selective inhibitors of Cox-2 bind to and inactivate Cox-2 by a noncovalent, time-dependent, irreversible mechanism (118). Included in this group of agents are NS398, DuP697, and SC58125, which block the synthesis of PGs responsible for inflammation without limiting basal production of PGs that are protective for gastric mucosa and renal blood flow (119–121). A recent, endoscopically controlled study showed that a selective inhibitor of Cox-2 caused much less injury to the mucosa of the upper gastrointestinal tract than a classical NSAID (122). Besides the theoretical safety advantages of treating inflammatory disorders with selective inhibitors of Cox-2, these agents may prove useful for preventing and treating a variety of cancers. Extensive testing of selective inhibitors of Cox-2 is now underway in a range of experimental models of carcinogenesis (123).

Inhibition of Cox-2 Expression and Cancer Prevention

The signal transduction pathways and transcription factors that mediate the induction of Cox-2 provide additional important sites for selective downregulation of the function of Cox-2 (Fig. 5).

Enhanced tyrosine kinase activity is observed in many

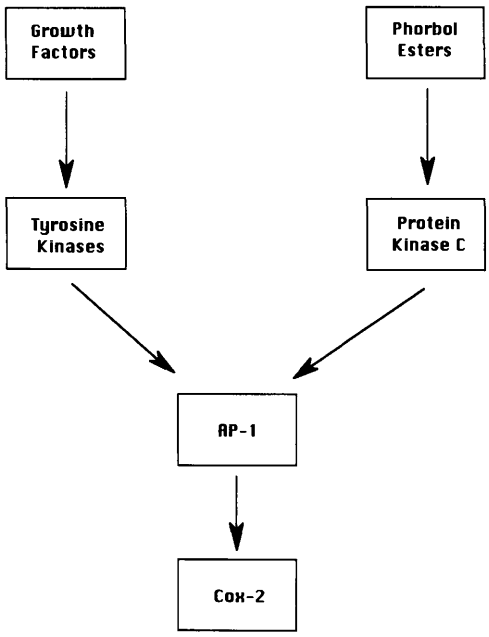

Figure 5. Schematic for regulation of Cox-2 gene expression *via* AP-1 transcription factor. The scheme is not complete; other components can regulate the gene, and pathways represented by arrows are more complex than shown. Arrows, upregulation of target. Note, however, that inhibitors of tyrosine kinases, PKC, and AP-1 transcription factors could be chemopreventive agents by inhibiting expression of the Cox-2 gene.

cancers (124); and deregulated signaling of intracellular tyrosine kinases can promote carcinogenesis (125). Hence, tyrosine kinases and their signaling pathways have been identified as potential targets for chemopreventive drug design. Such therapy is likely to work, in part, by downregulating the expression of Cox-2, because Cox-2 is induced by numerous agents that activate tyrosine kinase signaling, such as EGF, PDGF, Src, and Ras. For example, radicicol, a fungal antibiotic that inhibits $p60^{v\text{-}src}$ tyrosine kinase inhibits the expression of Cox-2 (126). Other naturally occurring inhibitors of tyrosine kinase activity such as genistein and herbimycin A also can inhibit Cox-2 expression (127). These data suggest that specific, tumor-related pathways for upregulating Cox-2 might be blocked selectively.

Tumor-promoting phorbol esters induce Cox-2 gene expression by activating the PKC pathway (26, 28, 128) (Fig. 5). A downstream target of activated PKC is the AP-1 transcription factor complex (129); AP-1 heterodimers are recognized to be important for activating Cox-2 promoter activity (31). Multiple PKC inhibitors, including staurosporine and calphostin C, are potential inhibitors of Cox-2 expression on this basis. Curcumin is another chemopreventive agent that inhibits the PKC pathway and AP-1–mediated gene expression (130). In preliminary studies, we have shown that curcumin, an active ingredient in turmeric (131), inhibits phorbol ester–mediated induction of Cox-2.

Retinoids, both naturally occurring and synthetic analogs of vitamin A, suppress carcinogenesis in various tissues by stimulating differentiation, apoptosis, and immune rec-

ognition of aberrant cells (132). Retinoids elicit their biological effects, in part, by blocking AP-1 mediated gene expression (133), which predicts that retinoids will downregulate phorbol ester–mediated induction of Cox-2. We have shown, in fact, that all-*trans* retinoic acid, 13-*cis*-retinoic acid, and retinyl acetate suppress phorbol ester– and epidermal growth factor–mediated induction of Cox-2 and PG production (128, 134). These results are likely to be important for understanding the anticancer properties of retinoids.

As with NSAIDs, toxicity is a serious limiting factor for the routine use of retinoids as chemopreventive agents, but the newly developed receptor-specific retinoids and AP-1–selective retinoids (135) may prove less toxic than classical retinoids. It will be important to investigate whether these compounds inhibit Cox-2 expression and possess anticancer properties.

Combination Therapy and Cancer Prevention

The material reviewed above shows that there is a significant relationship between levels of Cox-2 and cancer, and that Cox-2 is an excellent target molecule by which the incidence of cancer might be modulated. It already is known too that nonselective inhibitors of Cox-1 and Cox-2 are effective chemoprotective agents, but their use for this purpose has been limited because of side effects, including peptic ulcer disease and nephrotoxicity (48–51). It is now clear, however, that Cox-1 and Cox-2 have sufficiently different NSAID-binding sites (77, 78), that Cox-2 activity can be inhibited selectively (116), and that the gastric (122) and renal toxicity of NSAIDs is likely to be reduced by selective inhibition of Cox-2.

On the other hand, whereas selective inhibitors of Cox-2 will undoubtedly enhance the safety profile of NSAIDs, it is important to recognize the potential limitations of this single type of treatment for preventing cancer. The efficacy of an NSAID as a chemopreventive agent is likely to depend on its tissue availability. NSAIDs may be more effective, for example, in preventing colonic tumors than breast or brain tumors because of differences in bioavailability. Moreover, aside from problems relating to bioavailability, cellular transformation enhances transcription of Cox-2 (32, 33). Consequently, an overabundance of Cox-2 enzyme could be synthesized in transformed cells, which could override the positive therapeutic effects of direct NSAID-dependent inhibition of enzyme. Finally, whereas NSAIDs inhibit the production of PGs, most do not inhibit the peroxidase activity of Cox, which can generate proximate carcinogens from PAHs. Hence, upregulation of Cox-2, as a consequence of chronic inflammation or cellular transformation, may drive mutagenesis despite administration of NSAIDs.

In theory, it should be possible to overcome these potential limitations of NSAID therapy by using compounds (e.g., retinoids) that downregulate expression of the Cox-2 gene selectively. Because the Cox-1 and Cox-2 genes are separate, it is possible not only to inhibit Cox-2 selectively at the level of the enzyme but also to inhibit selectively the expression of the Cox-2 gene. Therefore, an important and novel chemopreventive approach would be to combine inhibitors of the pertinent signal transduction pathways that govern Cox-2 gene expression with specific inhibitors of Cox-2 activity. This type of combination therapy would limit the synthetic function of Cox-2 and the synthesis of proximate carcinogens *via* the peroxidase activity of the enzyme. A chemoprevention regimen combining a selective inhibitor of Cox-2 with a compound that suppressed the expression of Cox-2 is likely to be more effective than either agent alone.

Future Directions

Although considerable progress has been made in understanding the relationship between Cox and tumorigenesis, numerous questions remain unanswered. Some of these questions and issues are described below.

1. More needs to be understood about the relative significance of different Cox-mediated effects in carcinogenesis. For example, overexpression of Cox-2 inhibits apoptosis (97) and increases the invasiveness of malignant cells (98), but the importance of these effects compared with the immunosuppressive effects (100–107) of PGs is unknown. Additionally, carcinogenesis is a multistage process. The relative importance of Cox as a target for chemopreventive agents during the different stages of carcinogenesis requires clarification.

2. Increased levels of PGs are detected in tumors (1–9). The relative contribution of epithelial and nonepithelial components to PG production is unclear, however, and must be defined. Additionally, it will be important to elucidate the effects of cellular transformation on phospholipase activities because inhibiting the release of arachidonate from membrane phospholipids also could be a useful approach to cancer prevention. A related question is whether combined inhibition of phospholipases and Cox will offer therapeutic advantage over agents that inhibit only Cox.

3. Since selective inhibitors of Cox-2 are likely to prove safer than compounds that inhibit Cox-1 and Cox-2, it becomes feasible to use inhibitors of Cox-2 in subjects at low to moderate risk for tumor formation. We need to know then whether selective inhibitors of Cox-2 suppress the formation of adenomatous polyps of the colon. These agents also must be studied to determine their efficacy for preventing other cancers including breast cancer and adenocarcinoma in patients with Barrett's esophagus.

4. In addition to chemoprevention, inhibitors of Cox could be useful for treating cancer. Selective inhibitors of Cox-2 are especially interesting drugs in this context because Cox-2 is the isoform upregulated in cancer (34–36, 52, 53). Moreover, because selective inhibitors of Cox-2 are likely to be much safer than classical NSAIDs, it should be possible to use these drugs over prolonged periods of time.

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