

## NSAIDs Inhibit Tumorigenesis, but How?

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### Abstract

Numerous epidemiologic studies have reported that the long-term use of nonsteroidal anti-inflammatory drugs (NSAID) is associated with a significant decrease in cancer incidence and delayed progression of malignant disease. The use of NSAIDs has also been linked with reduced risk from cancer-related mortality and distant metastasis. Certain prescription-strength NSAIDs, such as sulindac, have been shown to cause regression of precancerous lesions. Unfortunately, the extended use of NSAIDs for chemoprevention results in potentially fatal side effects related to their COX-inhibitory activity and suppression of prostaglandin synthesis. Although the basis for the tumor growth-inhibitory activity of NSAIDs likely involves multiple effects on tumor cells and their microenvironment, numerous investigators have concluded that the underlying mechanism is not completely explained by COX inhibition. It may therefore be possible to develop safer and more efficacious drugs by targeting such COX-independent mechanisms. NSAID derivatives or metabolites that lack COX-inhibitory activity, but retain or have improved anticancer activity, support this possibility. Experimental studies suggest that apoptosis induction and suppression of  $\beta$ -catenin-dependent transcription are important aspects of their antineoplastic activity. Studies show that the latter involves phosphodiesterase inhibition and the elevation of intracellular cyclic GMP levels. Here, we review the evidence for COX-independent mechanisms and discuss progress toward identifying alternative targets and developing NSAID derivatives that lack COX-inhibitory activity but have improved antineoplastic properties. *Clin Cancer Res*; 20(5); 1104–13. ©2013 AACR.

### Introduction

Despite significant advances in early diagnosis and the development of molecularly targeted drugs, cancer remains the leading cause of mortality in the Western world (1). Chemoprevention using pharmaceuticals or by dietary intervention represents a well-accepted approach to inhibit disease progression in individuals with precancerous lesions, and in high-risk populations with genetic predispositions or long-term exposure to environmental carcinogens such as cigarette smoke. However, the implementation of chemoprevention strategies mandates exceptional safety and efficacy. Over the past three decades, epidemiologic, clinical, and experimental studies have established that nonsteroidal anti-inflammatory drugs (NSAID) inhibit carcinogenesis in various tissues and at different stages of progression. Despite the strong evidence of activity, the use of NSAIDs for cancer chemoprevention is not recommended because of potentially severe gastrointestinal, renal, and cardiovascular side effects that result from COX

inhibition and the suppression of physiologically important prostaglandins. In addition, the chemopreventive efficacy of NSAIDs is incomplete, although it is unclear if this shortfall is due to dosage limitations or resistance factors.

The molecular and cellular mechanisms responsible for the cancer chemopreventive properties of NSAIDs are complex and likely involve multiple effects on cancer cells and their microenvironment. Inhibition of COX is generally thought to be the primary mechanism responsible for their antineoplastic activity, although numerous studies have concluded that alternative targets may be involved, as reviewed previously (2–4). Given that the use of NSAIDs for cancer chemoprevention is limited by COX-dependent toxicities, identifying the relevant targets that mediate their antitumor properties provides an opportunity to develop safer and more efficacious derivatives, or new chemical entities. In this review, we provide an overview of the chemopreventive effects of NSAIDs, highlight evidence that the mechanism involves COX-independent effects, and discuss progress toward identifying new targets and developing NSAID derivatives that lack COX-inhibitory activity.

### Classification of NSAIDs

NSAIDs are a chemically diverse family of drugs available over-the-counter or by prescription and are commonly used for the treatment of inflammation, pain, or fever. Their anti-inflammatory activity is attributed to the inhibition of COX (5) enzymes that catalyze the conversion of arachidonic acid into prostaglandin H<sub>2</sub>, the precursor for the synthesis of

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prostaglandins (PG), prostacyclin, and thromboxane A<sub>2</sub>—collectively referred to as eicosanoids. The three major PG products of COX activity, PGE<sub>2</sub>, PGD<sub>2</sub>, and PGF<sub>2α</sub>, promote inflammation, pain, and fever. Vane was the first to show that aspirin inhibits inflammation by suppressing PG synthesis (6), whereas COX inhibition was later shown to be responsible for this effect (7). Aside from their role in inflammation, eicosanoids are critically important for the homeostatic maintenance of the gastrointestinal mucosa, blood clotting, regulation of blood flow, and kidney function.

Two distinct isoforms of COX, COX-1 and COX-2, have been reported (8). COX-1 is constitutively expressed in most tissues, whereas COX-2 is induced by inflammatory stimuli, mitogens, or growth factors, and is generally associated with pathologic processes (9). Conventional NSAIDs, such as aspirin, ibuprofen, sulindac, and indomethacin, inhibit both COX-1 and COX-2, although aspirin has a unique mechanism involving irreversible acetylation of a serine residue in the catalytic domain of both enzymes (10). The recognition that COX-2 is the main mediator of inflammation led to the development of a new class of inhibitors with COX-2 selectivity (coxibs) to circumvent gastrointestinal and renal toxicities associated with nonselective NSAIDs. However, coxibs were later found to increase the risk of heart attack and stroke (11, 12), which resulted in the recognition that all NSAIDs have risks of cardiovascular side effects.

## Cancer Chemopreventive Properties of NSAIDs

### Epidemiologic and clinical evidence

Many population-based studies have concluded that long-term use of NSAIDs is associated with a lower risk of developing colonic adenomatous polyps and lower incidence of colorectal cancer (13, 14). Although fewer epidemiologic studies have been conducted in cancers other than colorectal cancer, most have reported an inverse correlation between the long-term use of NSAIDs and incidence of tumors of the breast (15, 16), lung (17), prostate (18), bladder (19), ovary (20), esophagus (19), and stomach (19).

Clinical evidence of activity for the treatment of precancerous conditions was first reported in case studies by Waddell and Loughry in 1983, in which administration of sulindac reduced colonic adenomas in patients with familial adenomatous polyposis (FAP; ref. 21). Later, three randomized clinical trials confirmed that sulindac at a daily dose of 300 to 400 mg reduced adenomas in patients with FAP by an estimated 71% within 4 to 6 months of treatment (22). By comparison, the COX-2 selective inhibitor celecoxib at an 800 mg daily dose decreased rectal adenomas in patients with FAP by only 28.0% after 6 months of treatment (23), which nonetheless led to the U.S. Food and Drug Administration (FDA) approval of celecoxib for the treatment of FAP in 1999. The anticancer activity of COX-2 inhibitors also sparked considerable interest in the role of COX-2 in carcinogenesis. However, subsequent studies in patients with sporadic adenomas using another COX-2

inhibitor, rofecoxib, revealed unexpected cardiovascular toxicity (24) that caused it to be withdrawn from the market and essentially halted other clinical trials of coxibs for cancer chemoprevention.

Several studies have also reported that NSAIDs reduce the risk of death in patients with advanced colon and breast cancers, and may prevent metastasis of primary tumors or reduce mortality after diagnosis of malignant disease (25, 26). One clinical study reported that indomethacin can significantly extend survival of patients with metastatic disease (27), which suggests that NSAIDs can inhibit biologic processes associated with tumor cell invasion.

### Evidence from experimental studies

The epidemiologic evidence that NSAIDs reduce the risk of developing cancer is supported by an abundance of reports from experimental animal models, including carcinogen-induced or transgenic models of colorectal, breast, and other types of cancer. Among the first reports of the anticancer activity of NSAIDs in rodent models are studies by Pollard and Luckert and Narisawa and colleagues that described the inhibitory effects of indomethacin on carcinogen-induced intestinal tumors (28, 29). Subsequent studies demonstrated antitumor efficacy for NSAIDs from different classes against colorectal carcinogenesis (30, 31). Many of these studies used the rodent azoxymethane carcinogen model, which closely mimics human colorectal cancer with mutations in β-catenin and adenomatous polyposis coli (APC; refs. 32, 33). Consistent with their benefits for the treatment of FAP, NSAIDs and COX-2 inhibitors are also effective in the Min mouse, which harbors the same germline mutation in the APC gene (34, 35). Notably, NSAIDs were found to strongly inhibit the formation of aberrant crypt foci (ACF), the earliest detectable neoplastic lesions in the colorectum (36, 37). Although most studies have reported that NSAIDs inhibit tumorigenesis if administered before azoxymethane exposure, studies by Reddy and colleagues established that NSAIDs are still highly effective when treatment is initiated later in tumor progression when ACF and adenomas already existed (38, 39). These observations are consistent with the ability of NSAIDs such as sulindac to cause the regression of existing lesions in patients with FAP (40).

## COX-Independent Mechanisms of NSAID Chemoprevention

Observations that certain eicosanoids, such as PGE<sub>2</sub>, are elevated in various human tumor tissues (41) and can stimulate tumor cell proliferation (42), along with studies implicating COX-2 in tumor progression (43) and regulation of apoptosis (44), led to the widely accepted belief that COX-2 is an important target responsible for the chemopreventive effects of NSAIDs. However, numerous studies challenge this assumption by providing evidence that these effects can be exerted through a COX-independent mechanism. For example, *in vitro* studies have demonstrated that NSAIDs inhibit proliferation and/or induce apoptosis in

multiple tumor cell lines of different origins irrespective of COX-1 or COX-2 expression (45, 46). In addition, the growth-inhibitory activity of NSAIDs cannot be reversed by PG supplementation (47). There is also a discrepancy between the potency of a particular NSAID to inhibit COX-1 and/or COX-2 and its potency to inhibit tumor cell growth, whereby the concentration required to inhibit tumor cell proliferation is much higher than that required to inhibit COX activity, as shown in Table 1. This is an important consideration because experimental and clinical studies typically demonstrate chemopreventive efficacy of NSAIDs at doses appreciably higher than those necessary for anti-inflammatory effects. For example, a clinical trial of celecoxib involving FAP patients showed that a supratherapeutic dosage of 400 mg twice daily of celecoxib caused a mean reduction in colorectal polyp count of 28.0%, which was significantly greater than the 4.5% reduction observed with placebo ( $P < 0.005$ ; ref. 23). However, the recommend anti-inflammatory dosage of 100 mg of celecoxib twice daily only caused a 11.9% reduction, which was not statistically different from that for placebo ( $P = 0.33$ ). The possibility that an off-target effect accounts for the chemopreventive activity of NSAIDs may therefore explain their incomplete efficacy in clinical trials involving standard anti-inflammatory dosages.

Perhaps the strongest evidence for a COX-independent mechanism comes from experimental studies showing that non-COX-inhibitory metabolites (48), enantiomers (49), or derivatives (50) retain or have improved antitumor activity compared with the parent NSAID. Among these, the sulfone metabolite of sulindac, exisulind, is the most studied, for which there is an abundance of evidence of efficacy from various rodent models of carcinogenesis (51–53), as summarized in Table 2. Figure 1 illustrates the metabolism of sulindac into the active sulfide form and the non-COX-inhibitory sulfone. In addition, exisulind has been reported to inhibit tumor cell growth and induce apoptosis in multiple tumor types despite lacking COX-1- or COX-2-inhibitory activity (48). In studies involving the azoxymethane model of rat colon tumorigenesis, exisulind inhibited tumor

formation at dosages that did not reduce prostaglandin levels in the colon mucosa and achieved plasma concentrations above those required to inhibit tumor cell growth and induce apoptosis *in vitro* (52). In clinical trials, exisulind displayed significant adenoma regression in patients with familial (54) or sporadic (55) adenomatous polyposis but did not receive FDA approval due to hepatotoxicity and because of inherent problems with disease variation among patients with FAP that were encountered during the registration trial. Nonetheless, its strong chemopreventive activity in preclinical models supports the importance of COX-independent mechanisms and the rationale for developing other non-COX-inhibitory sulindac derivatives with improved potency and target selectivity.

### Molecular Targets

Although an NSAID may act upon a COX-independent target with relatively high specificity, it is generally recognized that a combinatorial action on multiple pathways through direct molecular targets as well as epigenetic and posttranscriptional mechanisms is responsible for the chemopreventive properties of NSAIDs. Some of the major pathways targeted by NSAIDs are discussed below and summarized in Table 3.

### Induction of apoptosis

NSAIDs have long been recognized to inhibit tumor cell growth in cell culture models with significantly different potencies across chemical families (56). The basis for this activity was first reported to involve apoptosis induction by two independent groups in 1995 (57, 58). The mechanism seemed to be unrelated to COX inhibition as evident by the ability of exisulind to also induce apoptosis. Apoptosis emerged as the major mechanism of NSAID chemoprevention following observations that treatment with sulindac can stimulate apoptosis in the normal rectal mucosa of patients with FAP (59), in normal intestinal mucosa of *APC<sup>Min</sup>* mice (60), and in the colorectal carcinomas of carcinogen-treated rats (61). In addition, exisulind was

**Table 1.** Potency of a panel of NSAIDs to inhibit colon tumor cell growth and COXs

NSAID	Growth IC <sub>50</sub> <sup>a</sup> (μmol/L)	COX-1 IC <sub>50</sub> <sup>b</sup> (μmol/L)	COX-2 IC <sub>50</sub> <sup>b</sup> (μmol/L)	Serum levels (μmol/L) <sup>c</sup>
Celecoxib	50	>30	2.25	2
Sulindac sulfide	60	1.02	10.4	15
Diclofenac	160	0.14	0.05	6
Indomethacin	180	0.16	0.46	1.4
Piroxicam	900	0.76	8.9	17
Ibuprofen	975	4.75	>30	40
Flurbiprofen	1,800	0.44	6.42	53
Aspirin	5,000	4.5	13.9	10

<sup>a</sup>HT-29 human colon tumor cells, 72-hour MTS assay (106).

<sup>b</sup>Whole blood COX assays (107).

<sup>c</sup>From therapeutic dosages (108).

Table reproduced from Gurpinar et al. (106).

**Table 2.** Chemopreventive efficacy of sulindac sulfone (exisulind) in rodent models of carcinogenesis

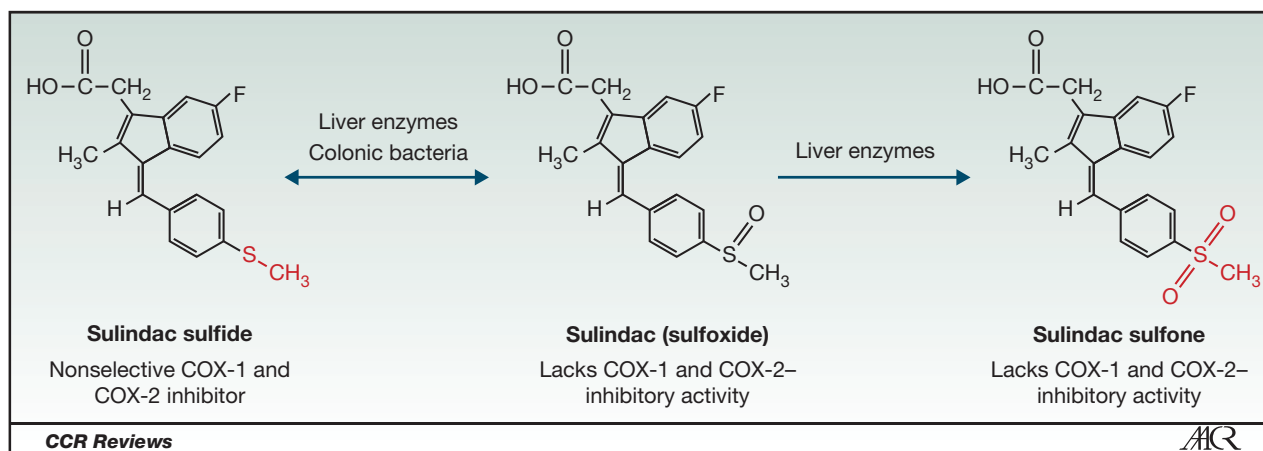
Model	Species	Dosage	Efficacy	Reference
Colon	Rat	1,000–2,000 ppm	69%–81%	(52)
Colon	Rat	600–1,200 ppm	41%–83%	(109)
Colon (ACF)	Rat	20 mg/kg bid	31%	(110)
Colon (ACF)	Rat	1,000–2,000 ppm	42%–37%	(111)
Mammary	Rat	300–600 ppm	44%–50%	(53)
Lung	Mouse	250–750 ppm	32%–82%	(51)
Bladder	Rat	800–1,200 ppm	36%–64%	(112)
Prostate	Rat	1,000 ppm	80%	(113)

reported to induce apoptosis in rectal polyps of patients with FAP but not in normal rectal mucosa, which implies an aspect of tumor selectivity (54). Consistent with these observations, studies using cell culture models demonstrate that NSAIDs, as well as their non-COX-inhibitory derivatives, can induce apoptosis in various cancer cell lines.

**Effects on Wnt/ $\beta$ -catenin pathway.** Dysregulation of Wnt signaling due to inactivating mutations in APC or activating mutations in  $\beta$ -catenin is involved in the development of multiple types of cancer, especially colorectal cancer (62). The efficacy of NSAIDs to inhibit polyp formation in patients with FAP and in  $APC^{Min}$  mice suggested that they may compensate for such mutations by inhibiting Wnt signaling. Studies have reported that sulindac can reduce nuclear  $\beta$ -catenin levels and induce  $\beta$ -catenin degradation, which could explain its antiproliferative and proapoptotic activity (63, 64). Similarly, both exisulind (65) and celecoxib (66) were reported to decrease  $\beta$ -catenin levels and inhibit the transcriptional activity of the  $\beta$ -catenin/T-cell factor/lymphoid enhancer factor complex. NSAIDs may therefore inhibit tumor cell growth by suppressing oncogenic  $\beta$ -catenin signaling through a COX-independent mechanism. Notably, colonic polyps of patients with FAP treated with sulindac show reduced nuclear accumulation

of  $\beta$ -catenin (67). Moreover, a recent study by Qui and colleagues showed that sulindac can selectively eliminate intestinal stem cells with nuclear or phosphorylated  $\beta$ -catenin and that aberrant Wnt signaling in  $APC^{Min}$  mice and in human colonic polyps is through the induction of apoptosis (68). These observations are corroborated by findings that sulindac downregulates  $\beta$ -catenin levels in hematopoietic progenitor cells, which carry oncogenic fusion proteins, resulting in reduced stem cell capacity and increased differentiation potential (69). These studies suggest that removal of cancer stem cells through direct inhibitory effects on Wnt/ $\beta$ -catenin signaling and induction of apoptosis is an important mechanism that mediates the chemopreventive effects of sulindac.

**Modulation of cGMP phosphodiesterase signaling.** Previous studies with exisulind suggested that cyclic guanosine 3',5'-monophosphate (cGMP) phosphodiesterase inhibition is an important COX-independent mechanism to suppress  $\beta$ -catenin signaling (65). In these studies, exisulind and several potent derivatives were found to inhibit cGMP phosphodiesterase activity and reduce oncogenic levels of  $\beta$ -catenin by increasing intracellular cGMP levels and activating cGMP-dependent protein kinase (PKG). Although exisulind displayed modest potency to inhibit



**Figure 1.** Metabolism of sulindac. Prodrug sulindac undergoes reversible reduction into the active sulfide form through the action of liver enzymes and colonic bacteria. Sulindac sulfide is a nonselective COX inhibitor and is responsible for the anti-inflammatory properties of sulindac. The sulfone metabolite is generated by irreversible oxidation of the sulfoxide in the liver and does not have anti-inflammatory activity. Figure adapted from Gulpinar et al. (106).

**Table 3.** COX-independent molecular targets of NSAIDs and their metabolites

	Sulindac Sulindac	Sulindac sulfide	Sulindac sulfone	Celecoxib	Aspirin	Salicylate	Indomethacin	R-etodolac	References
COX-1	—	x	—	—	x	—	x	—	
COX-2	—	x	—	x	x	—	x	—	
COX-independent targets									
cGMP PDE	—	x	x	x	—	—	—	—	(65, 71, 72, 114)
PPAR $\gamma$	—	—	—	—	—	—	x	—	(78)
PPAR $\delta$	—	x	—	—	—	—	x	—	(80)
RXR- $\alpha$	—	x	—	—	—	—	—	x	(81, 82)
IKK- $\beta$	x	—	—	—	x	x	—	—	(74, 75)
SERCA	—	x	—	x	—	—	—	—	(74, 87)
CA IX/XII	—	—	—	x	—	—	—	—	(83, 115)
Sp1	—	—	—	x	—	—	—	—	(91)
AMPK	—	—	—	—	x	x	—	—	(76, 77)
Gene expression									
NAG-1	—	x	x	—	x	x	x	—	(95, 97)
15-Lox-1	—	—	x	—	—	—	—	—	(116)

Table adapted from Gurpinar et al. (106).

phosphodiesterase and did not show evidence of selectivity for cGMP degrading isozymes, more recent studies with sulindac sulfide showed appreciably greater potency and selectivity to inhibit cGMP hydrolysis among several cGMP degrading isozymes, including PDE2, 3, 5, and 10 (70). Notably, studies showing an association between inhibition of the cGMP-specific PDE5 isozyme and the tumor cell growth-inhibitory activity of sulindac reinforce the importance of cGMP signaling (71). Moreover, the ability of PDE5 siRNA to mimic the selective nature by which sulindac induces apoptosis provides strong evidence for a role of the cGMP/PKG pathway in suppressing oncogenic  $\beta$ -catenin signaling. However, it is possible that additional or other cGMP PDE isozymes are involved because high concentrations of PDE5 inhibitors are required to inhibit tumor cell growth compared with concentrations required to inhibit purified PDE5. Other NSAIDs also inhibit cGMP phosphodiesterase activity, which in many cases matches their potency to suppress tumor cell growth (72).

PKG is thought to be the main kinase responsible for the antiproliferative and apoptosis-inducing activity of cGMP signaling. PKG activation attenuates  $\beta$ -catenin mRNA levels by directly inhibiting transcription from the *CTNNB1* gene (70) and by suppressing  $\beta$ -catenin nuclear translocation, possibly by inducing its sequestration by FOXO4 (73). These observations point to a mechanistic link between NSAID inhibition of cGMP phosphodiesterase and the suppression of Wnt signaling that is independent of COX binding, as illustrated in Fig. 2.

**Other targets.** Several additional molecules shown to be direct NSAID targets are particularly noteworthy. For example, studies provide evidence that aspirin and its deacetylated metabolite salicylate, as well as sulindac sulfide and exisulind, can inhibit NF- $\kappa$ B signaling (74, 75). Aspirin and salicylate were found to be ATP-competitive inhibitors of

IKK kinase- $\beta$  (IKK- $\beta$ ), the upstream positive regulator of NF- $\kappa$ B, suggesting that the antiapoptotic effects involve direct binding to IKK- $\beta$ . A recent report by Hawley and colleagues showed that salicylate can also bind and inhibit AMP-activated protein kinase (AMPK), a key protein kinase involved in the regulation of cellular metabolism and proliferation (76). These findings are consistent with a concomitant report by Din and colleagues, which showed that aspirin can activate AMPK in colon tumor cell lines and in the rectal mucosa of patients on a daily aspirin regimen (77) and suggest that AMPK may be an important target that mediates the chemopreventive effects of aspirin.

In addition, indomethacin, ibuprofen, and sulindac sulfide have all been reported to induce PPAR $\gamma$  promoter activity, the loss of which is implicated in colorectal carcinogenesis (78, 79). On the other hand, indomethacin and sulindac sulfide both can bind and repress transcriptional activity of PPAR $\delta$ , a growth-promoting protein activated by COX-2-derived prostacyclin (80). Furthermore, the *R*-enantiomer of etodolac, which lacks COX-inhibitory activity, has been shown to bind retinoid X receptor  $\alpha$  (RXR- $\alpha$ ) and selectively induce apoptosis in tumor cell lines (81). Sulindac sulfide was later demonstrated to specifically bind a truncated form of RXR- $\alpha$  expressed in cancer cells and lead to apoptosis through suppression of Akt signaling (82). In the same study, a sulindac derivative devoid of COX-inhibitory activity but with improved potency to bind RXR- $\alpha$ , K-80003, was shown to have significant antitumor activity *in vitro* and *in vivo*.

Several carbonic anhydrases (CA I, II, IV, IX, and XII) are inhibited by celecoxib in the low nanomolar range, at values significantly lower than its IC<sub>50</sub> for COX-2 inhibition (83). CAs are enzymes that regulate acid-base balance in tissues and are crucial for hypoxic adaptation in tumor cells. Their expression levels correlate with tumor aggressiveness and

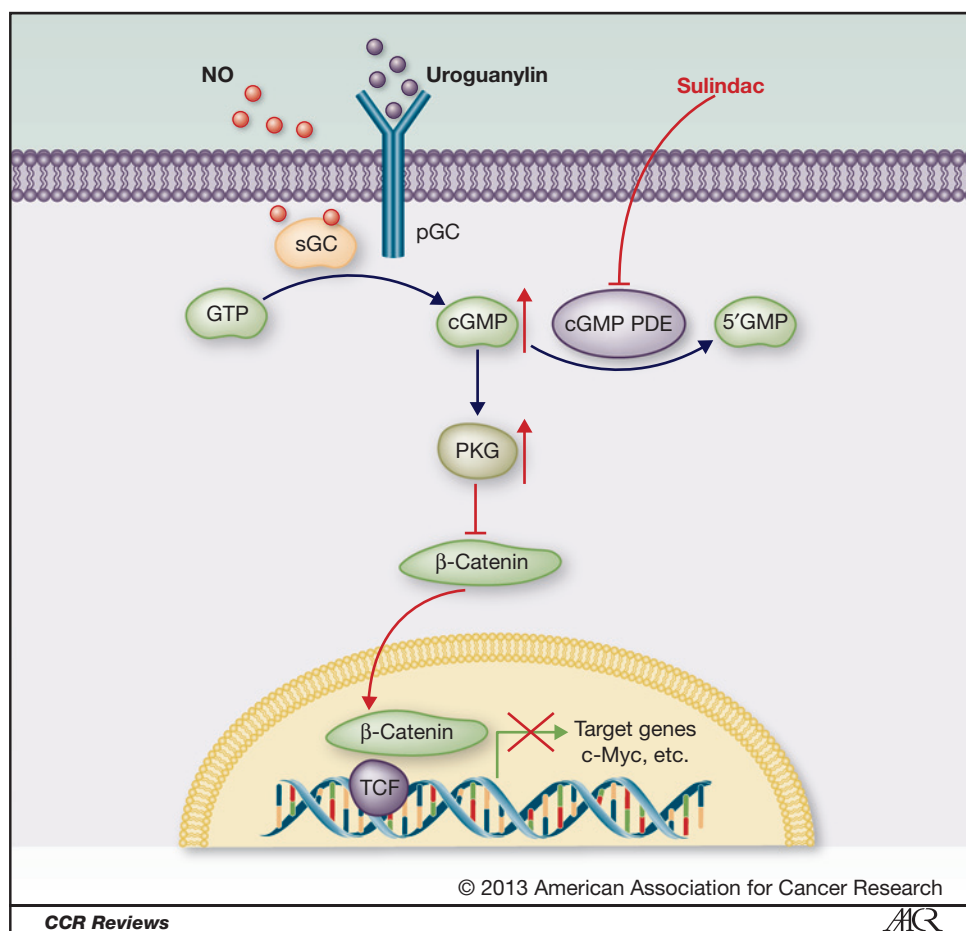


Figure 2. A mechanistic model of the cGMP/PKG pathway and the antineoplastic properties of sulindac.

a poor prognosis (84). Another direct target of celecoxib is the sarcoplasmic/ER  $\text{Ca}^{+2}$  ATPase (SERCA), which maintains the  $\text{Ca}^{+2}$  gradient between the cytosol and the estrogen receptor (ER). Binding of celecoxib, as well as its non-COX-inhibitory derivative dimethylcelecoxib, leads to rapid release of calcium from the ER, followed by activation of ER stress response and induction of apoptosis (85, 86). A more recent study has shown that sulindac sulfide can also bind SERCA in a similar fashion albeit with low potency (87).

#### Inhibition of angiogenesis and metastasis

NSAIDs, such as sulindac sulfide (88), exisulind (89), and celecoxib (90), have also been shown to inhibit angiogenesis and tumor cell invasion, although these observations are largely limited to the preclinical setting. It is plausible to suggest that the antiangiogenic properties of NSAIDs result from direct effects on endothelial cell survival and proliferation via the aforementioned targets, such as cGMP phosphodiesterases, IKK- $\beta$ , or SERCA. However, several other molecules involved in angiogenesis regulation have also been proposed to mediate these effects. For example, celecoxib can directly inhibit the DNA-binding activity of Sp1 transcription factor, a crucial driver of VEGF overexpression in cancer cells (91). In

addition, sulindac sulfide, exisulind, and celecoxib have all been shown to inhibit invasion through downregulation of matrix metalloproteins (MMP) 2 and 9 (92). These are the principal enzymes involved in degrading type IV collagen of the basement membrane, enabling endothelial cells to reach hypoxic tumors and cancer cells to invade adjacent tissue, leading to metastasis (93). Furthermore, a recent report provides evidence that sulindac sulfide can inhibit tumor cell invasion by suppressing Nf- $\kappa$ B-mediated transcription of microRNAs in human colon and breast cancer cell lines (94). Overall, these reports demonstrate that NSAIDs can attenuate angiogenesis and invasion through COX-independent pathways.

#### Effects on gene expression

NSAIDs have been reported to modulate the expression of various genes involved in the regulation of cell survival and proliferation. Multiple NSAIDs, including indomethacin, aspirin, and sulindac sulfide, were found to induce the expression of NSAID-activated gene (*NAG-1/GDF-15*) independent of COX inhibition in colorectal cancer cell lines (95). Although the precise biologic functions of NAG-1 are poorly understood, it is a member of the TGF- $\beta$  superfamily that exhibits proapoptotic and antitumorogenic activity in animal and cell culture models (96). A recent study by Wang

and colleagues found that NAG-1 is strongly induced in the liver of Min mice after sulindac treatment, suggesting that NAG-1 induction may contribute to the tumor-inhibitory effects of sulindac (97).

### Novel NSAID Derivatives

Several groups of investigators have synthesized derivatives using various NSAID scaffolds to reduce their COX-inhibitory activity, while improving potency to inhibit tumor cell growth. Our group developed a rational drug design approach to selectively block COX binding by substituting the negatively charged carboxylic acid moiety of sulindac sulfide, which is common to most NSAIDs and essential for COX binding via its interaction with positively charged moieties in the active site. One such derivative, referred to as sulindac sulfide amide (SSA), was found to have significantly higher potency to inhibit colon tumor growth compared with sulindac sulfide, despite lacking COX-1- or COX-2-inhibitory activity (98). With promising drug-like properties, SSA was shown to be highly effective in a colon tumor xenograft model alone and in combination with camptothecin. Other investigators have shown the ability of SSA to inhibit tumor formation in the transgenic adenocarcinoma of mouse prostate (TRAMP) model of prostate cancer (99). Recent studies have shown that SSA inhibits tumor cell growth primarily through the induction of autophagy via suppression of Akt/mTOR signaling (100). Sulindac sulfide mimicked these effects on Akt signaling and induced autophagy, but only at concentrations higher than those required to inhibit tumor cell growth, whereas apoptosis seemed to be the primary mechanism of cell death. Additional sulindac derivatives have since been developed, for example, that selectively inhibit PDE5 and have antitumor activity without inhibiting COX-1 or COX-2 (50). Recent efforts to develop improved chemopreventive agents also include the synthesis of phospho-derivatives that lack COX-inhibitory activity, such as phospho-sulindac and phospho-aspirin, but display high safety and efficacy in preclinical models of various cancer types (101, 102). Furthermore, the sulindac derivative K-80003 that selectively targets RXR- $\alpha$  (82) and celecoxib derivatives OSU-03012 (103) and dimethyl-celecoxib (104) that inhibit PDK-1 without COX inhibition represent other examples of separating COX-inhibitory activity and antitumor efficacy. These experimental agents demonstrate the feasibility of developing safer and more efficacious drugs for chemoprevention by chemically designing out COX binding while improving target selectivity. Moreover, these findings highlight the utility of NSAIDs as pharmacologic probes for target discovery, which could result in the development of new chemical entities with the potential for greater tumor selectivity.

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### Conclusions

Traditional NSAIDs and selective COX-2 inhibitors represent some of the most extensively studied agents with known chemopreventive activity. However, toxicities resulting from COX inhibition and incomplete efficacy limit their use for cancer chemoprevention. Currently, there are no approved therapies for the primary chemoprevention of FAP and preventive options are severely limited for high-risk individuals with precancerous lesions. A safe and efficacious chemopreventive drug can serve as an adjunct to surgery and prevent the formation of new lesions while reducing the overall risk of disease progression. However, further progress depends on increased understanding of the molecular mechanisms underlying the antineoplastic activity of NSAIDs. As summarized above, the inhibition of COX cannot explain all the observed chemopreventive effects of these drugs. Elucidating the involved targets and signaling pathways provides the opportunity to specifically target key molecules, select patient populations that are most likely to benefit from chemoprevention, and explain the underlying mechanisms of resistance. These studies will likely contribute to future chemopreventive strategies by enabling the identification of novel agents or guiding the modification of existing ones. Finally, using NSAIDs in combination with another chemopreventive or therapeutic agent represents an attractive strategy to increase efficacy and reduce toxicity. As established by a landmark phase III clinical study (105), sulindac is highly effective in combination with difluoromethylornithine for the prevention of sporadic colorectal adenomas in patients with a history of resected adenomas. Results from similar combination therapy trials can be put to immediate use given that NSAIDs are FDA approved and have a strong record of chemopreventive activity.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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**Conception and design:** E. Gurpinar, W.E. Grizzle, G.A. Piazza

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** G.A. Piazza

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** G.A. Piazza

**Writing, review, and/or revision of the manuscript:** E. Gurpinar, W.E. Grizzle, G.A. Piazza

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** W.E. Grizzle, G.A. Piazza

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