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Prevention of colorectal cancer through the use of COX-2 selective inhibitors

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Abstract Colorectal cancer is a major cause of morbidity and mortality accounting for an estimated 550,000 deaths annually worldwide. Colonic neoplasia develops in a stepwise fashion progressing from normal mucosa to adenomatous polyps to carcinoma, a process that takes years, thereby providing a prime opportunity for intervention. Although early detection by fecal occult blood testing and sigmoidoscopy can decrease the risk of cancer-related death by 20–30%, most persons never undergo appropriate screening. Population-based studies have previously determined that long-term ingestion of aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs) leads to a 40–50% reduction in mortality from colorectal cancer. These observational studies fueled subsequent mechanistic investigations that led to the identification of a molecular target, cyclooxygenase-2 (COX-2). COX-2 has tumor-promoting properties. Expression of COX-2 is virtually undetectable in normal intestinal mucosa, but is increased in approximately 50% of colonic adenomas and in 90% of colorectal carcinomas. Experimental studies in mice have revealed that genetic ablation or pharmacologic inhibition of COX-2 attenuates the number and size of intestinal polyps that develop in animals harboring a mutation in *Apc*, which confers an increased risk for intestinal neoplasia. Recent clinical studies using specific COX-2 inhibitors have shown that these compounds can: (1) reduce intestinal polyp burden in patients with familial adenomatous polyposis; (2) prevent the occurrence and/or recurrence of colorectal adenomas and cancers; and (3) negatively regulate angiogenesis in colorectal cancer liver metastases. Compared to nonselective NSAIDs, COX-2 specific inhibitors cause substantially fewer gastrointestinal side effects. These findings indicate that a widely used and relatively safe class of drugs may represent a viable and effective anticancer strategy for a disease that causes over a half-million deaths per year.

Keywords Colorectal cancer · Apoptosis · Angiogenesis · Polyps · Prostaglandins

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Introduction

Colorectal carcinoma is a major cause of mortality worldwide and accounts for nearly 4% of all deaths in industrialized countries [16, 31]. The incidence of this malignancy is clearly age-dependent and affects approximately 3 per 1000 persons over the age of 65 years [2, 11] (Fig. 1). Colon cancer progresses through a well-defined series of steps initiated by the transition from normal mucosa to adenomatous polyps, and finally to dysplasia and adenocarcinoma. The fact that this process takes several years affords prime opportunities for early detection and intervention, and it is widely accepted that removing polyps at an early stage prevents the progression to neoplasia. However, despite evidence that early detection by fecal occult blood testing and flexible sigmoidoscopy can decrease the risk of colorectal cancer mortality by 20–30%, most persons do not undergo appropriate screening [15].

An emerging trend in cancer research is rational drug design, which begins with a molecular target that is first identified in the laboratory. Investigations into chemoprevention of colon cancer, however, have proceeded in a reverse direction. The current emphasis on inhibition of cyclooxygenase-2 (COX-2) was initiated by astute clinical observations that persons ingesting aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs) on a regular basis have a 40–50% reduction in risk of

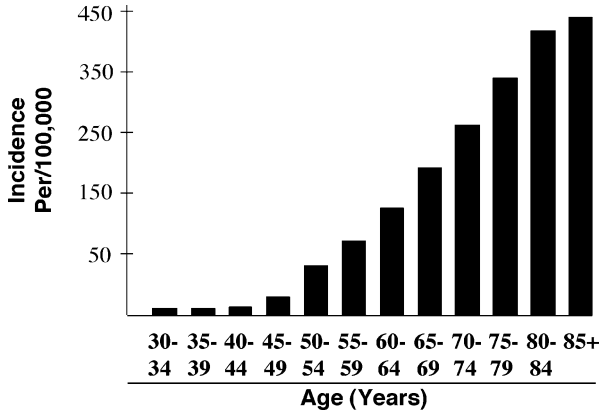

Fig. 1 Worldwide Incidence of colorectal cancer [2, 11]

mortality from colorectal cancer [13, 14, 49]. These observational studies formed the nidus for laboratory-based investigations that identified COX-2 as a molecule with tumor-promoting properties. A seamless transition from the bench back to the bedside has now occurred with recent data demonstrating that specific COX-2 inhibitors can reduce intestinal polyp burden in patients with familial adenomatous polyposis (FAP), prevent the occurrence and/or recurrence of colorectal adenomas and cancers, and negatively regulate angiogenesis in colorectal cancer liver metastases [10, 37, 45]. These results have raised the tantalizing possibility that a widely available and relatively safe class of pharmacologic agents may represent a viable preventive strategy for a disease that causes over 500,000 deaths annually worldwide. These observations also underscore the importance of continuing to identify mechanisms that promote colon carcinogenesis, which will ultimately enable physicians to appropriately focus diagnostic testing and prevention therapy.

NSAIDs reduce the risk of colorectal cancer

One of the first observations that NSAID use may be beneficial in the treatment of colon cancer was detailed in a report from Waddell and Loughry who observed a reduction in polyp burden in patients with FAP following ingestion of NSAIDs on a regular basis for pain relief [54]. This seminal paper stimulated a number of population-based studies designed to investigate if NSAIDs exerted protective effects on colon cancer. Results from both retrospective and prospective studies clearly indicated that continued ingestion of aspirin or other NSAIDs was associated with a 40–50% reduction in colon cancer risk [13, 14, 49]. The most compelling evidence that these medications can decrease the risk of polyp formation and cancer is derived from studies focused on patients with FAP. Three independent investigations have demonstrated that use of the NSAID sulindac significantly reduces the number and size of colonic polyps in FAP patients, and, when therapy is halted, this trend is reversed [12, 24, 34]. Thus, sulindac exerts biologically relevant effects on polyp regression in patients with an inherited predisposition for colorectal carcinogenesis.

There have also been numerous prospective randomized trials investigating the role of aspirin for prevention of colorectal polyps. Sandler and coworkers evaluated the effects of aspirin (325 mg daily) versus placebo on polyp burden in patients with a history of colorectal cancer and demonstrated a statistically significant reduction in the incidence of polyp development [40]. Baron and colleagues found a similar reduction in the risk of recurrent adenomatous polyps in patients treated with aspirin versus placebo [3]. A recent study performed by Benamouzig and coworkers evaluated more than 200 patients with a history of colorectal adenomas 1 year after treatment with two different doses of a soluble aspirin formulation (lysine acetylsalicylate) compared to placebo [4]. Both doses of aspirin significantly reduced the risk for recurrent adenomas [4]. Collectively, these studies indicate that aspirin causes a measurable reduction in colonic adenoma recurrence among high-risk patients.

Mechanisms for chemoprevention of colorectal cancer by NSAID medications

NSAIDs possess antiinflammatory properties via their ability to inhibit COXs [15]. These enzymes catalyze key steps in the conversion of arachidonic acid to endoperoxide (PGH_2), a substrate for a variety of prostaglandin synthases that, in turn, catalyze the formation of prostaglandins and other eicosanoids [15] (Fig. 2). Prostaglandins regulate a diverse array of physiologic processes including immunity, maintenance of vascular tone and integrity, nerve development and bone metabolism [15]. Three isoforms of COX have been identified to date, each possessing similar activities, but differing in expression characteristics and inhibition profiles by NSAIDs. COX-1 was purified in 1976 and is expressed constitutively in many cells and tissues [32, 58]. A second COX enzyme, COX-2, was later identified

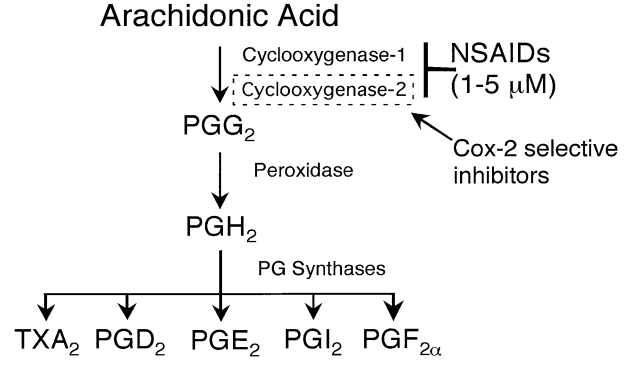

Fig. 2 COXs catalyze key steps in the prostaglandin pathway

and cloned by two independent groups [23, 44]. In contrast to COX-1, COX-2 expression was found to be inducible in cells transformed with the oncogene v-*src* or treated with phorbol esters [23, 61]. Subsequent studies have now shown that COX-2 can be induced by a variety of growth factors and proinflammatory cytokines in a number of pathophysiologic conditions [58].

The discovery of an inducible COX led to subsequent investigations designed to determine whether dysregulation of COX-2 expression affects colon carcinogenesis. COX-2 is overexpressed in the majority of colorectal cancers and adenomas when compared to normal adjacent colonic mucosa [9, 20, 41]. The most direct evidence implicating COX-2 in colon carcinogenesis has been obtained using genetic models of COX-2 deficiency. Oshima and colleagues examined intestinal polyp number in mice ($Apc^{\Delta 716}$ mice) engineered to express a truncated *Apc* gene that were then bred onto either a wildtype or a COX-2-null background [35]. The number and size of polyps was reduced in COX-2-deficient versus wildtype mice, and treatment of $Apc^{\Delta 716}$ COX-2 wildtype mice with a specific COX-2 inhibitor or sulindac also reduced polyp number [35]. Two independent groups have shown a similar reduction in tumor multiplicity using another mouse model of FAP (*Min*) treated with the nonselective NSAIDs sulindac or piroxicam [5, 19]. Reddy and coworkers also reported a 40% reduction in aberrant crypt formation in carcinogen-treated rats which were maintained on a selective COX-2 inhibitor [38].

There are additional genetic data implicating a role for COX-2 in carcinoma growth. When injected into COX-2-null mice, transformed lung carcinoma cells grow more slowly than when implanted into wildtype mice [59]. Liu and colleagues generated transgenic mice that express COX-2 under the control of the murine mammary tumor virus (MMTV) promoter/enhancer [26]. Although nulliparous mice did not develop tumors, multiparous mice demonstrated a significant increase in mammary-gland carcinomas compared to controls, indicating that overexpression of COX-2 per se is sufficient to induce cellular transformation [26]. Transgenic expression of COX-2 in basal keratinocytes similarly leads to epidermal hyperplasia and dysplasia [33]. Collectively, these studies implicate COX-2 as a tumor promoter in a variety of organ systems.

COX-independent effects of NSAIDs

Certain NSAIDs can alter cellular growth and death independently of their ability to inhibit COXs when administered at high doses [15]. On the basis of these results, several alternative targets have been proposed that may mediate the effects of NSAIDs on cellular responses. High doses of aspirin or sulindac can inhibit the kinase activity of Ikappa B kinase beta, which prevents nuclear factor-κB nuclear translocation [22, 62]. Another specific host pathway through which NSAIDs may influence cell growth and death are the peroxisome proliferator-activated receptors (PPARs). PPARα, PPARδ, and PPARγ constitute a family of nuclear hormone receptors with important roles in the regulation of fatty acid oxidation and glucose utilization [21, 60]. PPARs form functional heterodimers with the retinoid X receptor (RXR) family of nuclear receptors [21, 60], and it is now appreciated that these receptors are important in regulating pathways beyond energy homeostasis. For example, although PPARγ was originally identified as a transcription factor essential for adipocyte differentiation, there is now increasing evidence to indicate a role for this receptor in regulating other cell types including macrophages, lymphocytes, and epithelial cells [8, 46, 48]. Fatty acids and certain fatty-acid metabolites have been identified to be putative endogenous ligands for these receptors and PPARs have been proposed as direct targets for NSAIDs [15]. Indomethacin, a nonselective NSAID, binds to and induces PPARα and PPARγ transcriptional activity [25], while PPARδ is a direct target of sulindac [17]. NSAIDs have also been shown to reduce levels of *bcl-x*, an antiapoptotic gene, which increases apoptosis in colon cancer cell lines [63]. A concern with each of these studies, however, is that the NSAID concentrations required to induce cellular responses are in the 50–1000 μM range, which is nearly 200-fold higher than the concentrations of selective COX-2 inhibitors that are required to suppress tumorigenesis in rodent models of intestinal cancer.

Mechanisms through which COX-2 promotes tumorigenesis

Although the results described above suggest that NSAID therapy may suppress colon carcinogenesis via non-COX pathways, the doses required to exert such effects are extremely high, and thus the balance of in vivo and in vitro data overwhelmingly implicate a role for COX-2 in tumor development and progression. COX-2 overexpression in human and murine tumor samples is localized to both the epithelial cell compartment and/or the stroma (e.g. macrophages, fibroblasts, endothelial cells) [15]. This pattern of localization has raised intriguing hypotheses regarding mechanisms by which COX-2 metabolites may facilitate oncogenesis. Data that support epithelial cell autonomous effects of COX-2 include the following. Overexpression of COX-2 in intestinal epithelial cell lines confers resistance to apoptosis via increasing the level of the antiapoptotic protein Bcl-2 [51]. COX-2 upregulation also leads to increased cell migration and invasion that is accompanied by enhanced expression of several matrix metalloproteinases [52], and facilitates the formation of endothelial tubes which is associated with increased levels of angiogenic factors [53]. Conversely, transfection with COX-2 antisense cDNA reduces cellular proliferation [6].

The observation that cells adjacent to neoplastic epithelium also overexpress COX-2 forces consideration of the possibility that carcinogenesis may be directly

affected by the stromal compartment, and data exist to bolster this hypothesis. Inhibition of COX-2 blocks neovascularization in response to exogenous growth factors and this can be reversed by treatment with an agonist of the COX-2 metabolite, TxA_2 [7, 27, 28]. Similarly, corneal blood vessel formation in rats is suppressed by COX-2, but not COX-1, inhibitors [30]. Skin fibroblasts isolated from COX-2-null mice display defects in their ability to secrete several angiogenic factors compared to cells harvested from wildtype mice [59], suggesting that COX-2 may modify transformation by regulating the ability of fibroblasts to stimulate neovascularization.

Molecular mediators of the cellular effects of COX-2 in carcinogenesis

Prostaglandin metabolites of COX-2 exert their effects on cells by binding to G-protein-coupled receptors, which then alter cytosolic levels of cAMP and/or calcium [15, 47]. There are emerging data indicating that certain prostaglandins (PGI_2, PGD_2 metabolites) can also activate PPAR receptors and thus regulate gene expression [15]. PGE_2 has been reported to be increased in colon cancer tissue compared to normal colonic mucosa [39] and in vitro, this prostaglandin exerts procarcinogenic effects. For example, PGE_2 decreases apoptosis in colon cancer cell lines, which is accompanied by an increase in the antiapoptotic protein Bcl-2 [42]. Transformed colon cell lines also proliferate and migrate more rapidly when exposed to PGE_2, an effect that is likely mediated by binding of the PGE_2 receptor subtype EP4 [43]. PGE_2 has also been shown to induce hereregulin-B1, which increases cellular migration and invasion [1].

Genetic studies in mice also support the contention that prostaglandin signaling regulates tumor growth. Mice deficient in the prostaglandin receptor Ep1 are partially resistant to azoxymethane-induced aberrant crypt foci, and these changes are recapitulated in wildtype mice treated with an Ep1 antagonist [56, 57]. Further, *Min* mice with a genetic predisposition towards intestinal cancer exhibit a significant reduction in polyp burden following treatment with an antagonist of Ep1 [56]. Future studies of the role of specific prostaglandins and their cognate receptors using transgenic and knockout mice should more precisely define which synthases and receptors regulate specific points in the colorectal carcinogenic cascade.

Selective COX-2 inhibitors and prevention of colorectal neoplasia in human subjects

Numerous studies have demonstrated a significant reduction in risk and/or mortality from colorectal cancer in persons ingesting aspirin or other NSAIDs on a chronic basis. However, there are concerns regarding the long-term safety of these drugs in humans. Chronic aspirin and/or NSAID use results in an increased risk of gastrointestinal bleeding, even at relatively low doses, and side effects accelerate as populations age [58]. Based on these concerns, a new class of NSAIDs was designed that is highly selective for inhibition of COX-2, but which lacks the ability to inhibit the COX-1 isoform. Although these compounds were originally developed as antiinflammatory agents that could attenuate gastrointestinal side effects caused by concomitant COX-1 inhibition, they have the potential for use as chemopreventive agents [58]. This is primarily due to the fact that selective COX-2 inhibitors effectively inhibit tumor growth in animal models [15] (Table 1), and these encouraging results have stimulated recent studies in humans designed to address whether COX-2 inhibitors can slow or even reverse the development of colorectal cancer growth.

Steinbach and colleagues demonstrated that ingestion of celecoxib, a selective COX-2 inhibitor, reduces the number of colorectal adenomas in patients with FAP [45]. Rahme and coworkers performed a nested case-control study to evaluate the effects of exposure to selective or nonselective COX-2 inhibitors on the occurrence or recurrence of colorectal neoplasia in average-to-high-risk patients [37]. Exposure to at least 3 months of selective COX-2 inhibitors (rofecoxib or celecoxib) conferred a significant protective effect against colorectal adenomas and carcinomas, which is the first demonstration that agents specific for COX-2 protect against the development of these lesions in a general population [37]. Another recent clinical trial investigated the effects of rofecoxib on parameters of cell growth and death, and neovascularization in patients with colorectal cancer liver metastases [10]. Rofecoxib-treated metastases had a marginal decrease in microvessel density compared with placebo-treated tissue, but there were no differences in apoptosis or proliferation between the groups [10]. The dose of rofecoxib used (25 mg daily) was safe and well tolerated and significantly reduced ex vivo PGE_2 synthesis by metastatic

Table 1 Effects of COX-2 selective inhibitors in animal models of colorectal cancer

NSAID treatment	Outcome
Apc^{min} mouse	
Celecoxib	↓ polyp multiplicity
$Apc^{\Delta 716}$ mouse	
Rofecoxib	↓ polyp multiplicity
Azoxymethane-treated rat	
Celecoxib	↓ tumor incidence ↓ multiplicity
NS-398	↓ tumor incidence ↓ multiplicity
Nimesulide	↓ tumor incidence ↓ multiplicity
Nude mouse xenograft	
SC-58125	↓ colon carcinoma cell growth
Celecoxib	↓ colon carcinoma cell growth

tissue [10]. These results have set the stage for future studies that can more definitively evaluate the role of COX-2 inhibition in abrogating different points in the colorectal carcinogenic cascade.

Conclusions

The collective evidence from human, animal, and cell culture studies clearly indicates that targeted inhibition of COX-2 is a viable approach to colorectal cancer prevention and/or treatment. Future clinical studies assessing the ability of COX-2 inhibitors to prevent polyp formation will undoubtedly help to clarify the role of these compounds in cancer prevention. An exciting area of investigation is testing whether multimodality therapies that combine COX-2 inhibitors with drugs targeting additional cellular pathways can synergistically affect cancer occurrence or recurrence. Examples of this approach include combining selective COX-2 inhibitors with EGF receptor inhibitors and/or protein kinase A inhibitors [50], coupling inhibitors of COX-2 with compounds that block matrix metalloproteinases [55], combining specific COX-2 inhibitors with herceptin [29], or simply adding COX-2 inhibitors to standard colon cancer chemotherapy regimens.

Along with combinatorial approaches for treatment or prevention of spontaneous colon cancer, investigations focused on other types of colorectal cancer that may respond to COX-2 inhibition are areas ripe for exploration. For example, does inhibition of COX-2 affect the development of colorectal cancers that are associated with microsatellite instability? Persons with inflammatory bowel disease (IBD) have a significantly increased risk of colon cancer, and COX-2 has been reported to be increased in epithelial cells and monocytes within active foci of IBD [18, 36]. Does inhibition of COX-2, therefore, suppress the development of intestinal-type malignancies that arise within foci of chronic inflammation? Continued research on the role of COX-2 in colorectal carcinogenesis will undoubtedly permit novel treatment approaches to be identified for a disease that has few therapeutic options.

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