Cyclooxygenase-2 and Colorectal Cancer Chemoprevention: The β-catenin Connection

Maria Domenica Castellone,¹ Hidemi Teramoto,² and J. Silvio Gutkind¹

¹Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland and ²Kojin Hospital, Nagoya, Japan

Abstract

Colorectal cancer poses a major clinical challenge in the developed world where this disease is common. Recent findings suggest that the prostaglandin E₂, the proinflammatory product of elevated cyclooxygenase-2 activity in colon cancer, stimulates cancer cell growth through a G protein–dependent signaling pathway coupling the prostaglandin EP2 receptor to β-catenin control. These findings provide new insights into the molecular framework needed to evaluate chemopreventive strategies for colorectal cancer. (Cancer Res 2006; 66(23): 11085-8)

Background: Inflammation and Colon Cancer

Colorectal cancer is the third cause of cancer-related death in the Western world (1). The development of colon cancer results from the sequential accumulation of activating mutations in oncogenes, such as ras, and inactivating mutations, truncations, or deletions in the coding sequence of several tumor suppressor genes, including p53 and adenomatous polyposis coli (APC), together with aberrant activity of molecules controlling genomic stability (2). In particular, the loss of a functional APC protein is one of the earliest events occurring in sporadic colon cancer, suggesting that APC may act as a gatekeeper of the colonic epithelium (2). Indeed, germ-line mutations in a single allele of the APC tumor suppressor gene result in a hereditary condition, familial adenomatous polyposis (FAP), which is characterized by the presence of numerous colorectal polyps, some of which can progress to intestinal adenomas and eventually colorectal carcinomas on nutritional damage or loss of the wild-type allele (3). Similarly, mice harboring germ-line mutations in APC, Apcmin (multiple intestinal neoplasia) mice, are predisposed to the formation of intestinal adenomas (4). APC loss results in the cytoplasmic stabilization and nuclear translocation of β-catenin, whose transcriptional activity contributes to colon cancer progression (5). APC also plays a role in epithelial migration and can localize at kinetochores, thereby participating in chromosome segregation during mitosis (reviewed in ref. 6), which may contribute to the genetic instability and loss of epithelial polarity during the malignant transformation of colonic epithelia. However, in line with the central role of the β-catenin pathway in colorectal tumorigenesis, patients who do retain a functional APC harbor instead activating mutations in β-catenin or inactivating mutations in Axin (reviewed in refs. 2, 7). The latter is a large scaffold protein that binds APC and β-catenin, which inhibits the β-catenin pathway by forming a molecular complex with glycogen synthase kinase-3β (GSK-3β), a kinase that phosphorylates β-catenin and targets it for degradation by the proteosome (2, 7).

Chronic inflammation is now believed to play an important role in the development of numerous cancer types, including colon cancer (reviewed in ref. 8). For example, the use of nonsteroidal anti-inflammatory drugs (NSAID) drastically reduces the overall number and size of adenomas in patients with FAP, as well as in experimental animal models, such as the Apcmin mice (9), by the pharmacologic inhibition of two enzymes involved in prostaglandin biosynthesis, cyclooxygenase (COX)-1 and COX-2. Furthermore, healthy individuals using NSAIDs regularly can reduce by 40% to 50% their risk of developing colorectal cancers (10). Indeed, the accumulating clinical and experimental evidence now supports a potent antimutagenic efficacy of inhibiting COX-2 with NSAIDs as a chemopreventive strategy for colon cancer (reviewed in refs. 10, 11). Hence, these observations implicate the contribution of COX-2 and its metabolites, prostaglandins, in colon cancer development (11, 12).

COX-1 and COX-2 are key enzymes in the biosynthetic pathway by which arachidonic acid is first converted into an intermediate prostaglandin, PG-G2, and then metabolized to at least five structurally related prostaglandins, including prostaglandin E₂ (PGE₂), PGD₂, PGF₂α, PGI₂, and thromboxane A₂ by specific prostaglandin synthases (13). Whereas COX-1 is expressed at relatively constant levels in numerous tissues, the expression of COX-2 is normally low or absent in most cells and tissues but rapidly up-regulated by proinflammatory cytokines and tumor promoters (14). Thus, COX-2 represents the best target for therapeutic intervention in several disease states that involve chronic inflammation, including cancer (14). Increased levels of COX-2, but not COX-1, has been detected in 50% of colorectal adenomas and in up to 85% of colorectal cancer (15), and strong expression of COX-2 is a marker for poor survival (16). Among the COX-2-derived prostaglandins, PGE₂ has emerged as one of the most studied due to its direct role in inflammation and in the malignant progression of most solid tumors, including colon, breast, lung, head and neck, uterus, and stomach carcinomas (17). In particular for colon cancer progression, the direct procarcinogenic role of PGE₂ has been shown recently in a series of elegant animal model studies (18–20).

How the interplay between PGE₂ and APC-regulated pathways leads to colon cancer cell growth remains poorly understood. Ultimately, PGE₂ exerts its numerous biological functions, including the stimulation of cell migration, proliferation, tumor-associated neovascularization, modulation of immunosuppression, and inhibition of cell death, through the activation of its four cognate G protein–linked receptors, EP1 to EP4 (17, 21, 22). Detailed animal studies using gene knockout mice and recently
developed receptor subtype specific antagonists suggest that the prostaglandin receptors EP2, and to a lesser extent EP1 and EP4, they all play a role in colorectal cancer development (18–21).

**Convergent Pathways Link COX-2 and PGE\(_2\) to the Nucleus: The Emergent Role for \(\beta\)-catenin**

Whereas EP1 is coupled to G proteins of the Gq family and thus promote calcium mobilization and protein kinase C activation, EP2 and EP4 are Gs-coupled receptors and elevate the intracellular levels cyclic AMP (17). However, these second messenger-generating systems alone may not be able to explain the diversity of effects stimulated by PGE\(_2\) through EP receptors. Indeed, the mechanisms by which EP receptors promote their numerous functions, including colon cancer progression, are still under intense investigation. These receptors can promote the trans-activation of epidermal growth factor (EGF) receptor (EGFR) expressed in colon cancer cells, thereby initiating the activity of the EGFR signaling network (23, 24). How PGE\(_2\) receptors stimulate EGFR seems to involve an intracellular Src-mediated event (25), together with the overexpression and proteolytic release of the EGFR ligands amphiregulin and transforming growth factor-\(\alpha\) (TGF-\(\alpha\); refs. 23, 24).

On the other hand, as shown in Fig. 1, PGE\(_2\) can promote the proliferation of colon cancer cells through the activation of transcription factors not often linked to GPCRs, including the nuclear NR4A2 receptor, an orphan member of the nuclear receptor transcription factor superfamily that mediates the prosurvival effects of PGE\(_2\) (26), and peroxisome proliferator-activated receptor \(\delta\) (PPAR\(\delta\)), a member of the nuclear hormone receptor family.

![Figure 1](http://cancerres.aacrjournals.org/content/csr/66/23/11086/F1.large.jpg)
receptor family (27), whose gene deletion diminishes colon cancer development in Apc<sup>min</sup> mice, whereas its activation by PPAR<sub>γ</sub> agonists promotes adenoma growth in these colon-cancer-prone animals (28, 29).

In an interesting twist, recent studies have also revealed that PGE<sub>2</sub> can stimulate the β-catenin pathway and that activation of β-catenin is in turn necessary to stimulate cyclin D and e-myc, thereby promoting colon cancer cell growth (5, 30). The mechanism by which PGE<sub>2</sub> stimulates β-catenin is complex. The activation of G<sub>s</sub> by binding of PGE<sub>2</sub> to EP2 receptors provokes the release of Gi/γ subunits, which stimulate Akt through phosphatidylinositol 3-kinases (PI3K), whereas concomitantly G<sub>βγ</sub> binds directly to a structural region in Axin known as RGS domain. In certain cells, PGE<sub>2</sub>-stimulated protein kinase A (PKA) can activate Akt even further (31). These three prone mechanisms result in the release of GSK-3<sub>β</sub> from its complex with Axin and the phosphorylation and inactivation of GSK-3<sub>β</sub> by Akt (32). The inactivation of GSK-3<sub>β</sub> then leads to the stabilization and nuclear translocation of β-catenin, where it interacts with cofactors Tcf and Lef to activate transcription of genes that promote colon cancer cell proliferation (5). The ability of PGE<sub>2</sub> to stimulate β-catenin may not be limited to its G protein–coupled receptors, as leukotriene D<sub>4</sub> also stimulates β-catenin in colon cancer cells (33), and the potent mitogenic effect of lysophosphatidic acid (LPA) in colon cancer cells also requires the activation of β-catenin by its G protein–coupled receptors (GPCR) LPA-2 and LPA-3 (34). Of interest, PPAR<sub>γ</sub> is a direct transcriptional target of β-catenin (35), thus suggesting that the two most prominent nuclear events stimulated by PGE<sub>2</sub> may be interconnected, a possibility that warrants further investigation.

Colorectal Cancer Chemoprevention: Novel Molecular Targets?

Although a large body of evidence from clinical trials and population-based studies indicates that the use of NSAIDs is associated with a reduced risk of colorectal cancer, the prolonged use of nonselective NSAIDs is associated with gastrointestinal toxicity, due to the inhibition of the COX-1 isoform, an important housekeeping gene of the gastrointestinal system. Histamine 2 receptor antagonists and proton pump inhibitors can reduce this toxicity (36), but most efforts have focused on developing specific inhibitors of the COX-2 enzyme. Unfortunately, recent long-term trials have shown that certain COX-2-selective inhibitors can lead to increased cardiovascular morbidity, even if these side effects are dependent on the daily dose, length of treatment, and individual factors predisposing to cardiac risk (10). While toxic side effects may be acceptable, and almost inevitable, in the treatment of advanced cancers as with chemotherapy, they are not tolerated in chemoprevention (37). In this regard, for instance, individuals carrying highly penetrant genetic mutations, such as the FAP patients, can risk developing some adverse side effects; healthy populations, even those at high epidemiologic risk of developing colon cancer, cannot (38).

These side effects associated with long-term COX-2 inhibition result predominantly from the fact that general suppression of prostacylin production, which includes the protumorigenic PGE<sub>2</sub>, also leads to the reduced release of anti-thrombotic prostaglandins, such as PG-I<sub>2</sub>. A possible way to selectively block PGE<sub>2</sub> is to inhibit prostaglandin E synthase (PGES; ref. 39). Selective inhibitors of this enzyme, which is overexpressed in cancers (40), may be more effective and yield higher therapeutic ratios than COX inhibitors. Moreover, it would be important to determine the role of the recently described G<sub>α<sub>q</sub></sub>-β-catenin and NBA2 pathways and their interplay with APC in the chemopreventive effects of NSAIDs, as modulating these proteins with specific molecular inhibitors could provide new chemopreventive strategies in colorectal cancer. Molecular targeting has an important role in the prevention of cancer, as well as in its treatment. Among others, the most promising results come from the Food and Drug Administration approval in 1998 of tamoxifen as chemopreventive agent in women with increased risk of breast cancer and of celecoxib in 2000 as an adjunctive drug for individuals with FAP (41). In time, our increasing understanding of the molecular mechanisms involved in cancer development may ultimately afford the opportunity of developing better molecular-targeted drugs, with minimal side effects, which together with healthier choices of nutrition and lifestyles, will help in reducing cancer incidence.

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References


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