Molecular Pathways

Cyclooxygenase-2 and Cancer Treatment: Understanding the Risk Should Be Worth the Reward

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Abstract

Targeting the prostaglandin (PG) pathway is potentially a critical intervention for the prevention and treatment of cancer. Central to PG biosynthesis are two isoforms of cyclooxygenase (COX 1 and 2), which produce prostaglandin H₂ (PGH₂) from plasma membrane stores of fatty acids. COX-1 is constitutively expressed, whereas COX-2 is an inducible isoform upregulated in many cancers. Differences between COX-1 and COX-2 catalytic sites enabled development of selective inhibitors. Downstream of the COX enzymes, prostaglandin E₂ synthase converts available PGH₂ to prostaglandin E₂ (PGE₂), which can stimulate cancer progression. Significant research efforts are helping identify more selective targets and fully elucidate the downstream targets of prostaglandin E₂-mediated oncogenesis. Nonetheless, as a key rate-limiting control point of PG biosynthesis, COX-2 continues to be an important anticancer target. As we embark upon a new era of individualized medicine, a better understanding of the individual risk and/or benefit involved in COX-2 selective targeting is rapidly evolving. This review endeavors to summarize developments in our understanding of COX-2 and its downstream targets as vital areas of anticancer research and to provide the current status of an exciting aspect of molecular medicine.

Background

Prostaglandins: A seminal discovery. Proinflammatory lipids play a central role in cancer progression (1) and prostaglandins (PG) are among the most active of these molecules. As namesake products of the prostate gland, PGs were first isolated from seminal fluid (2), and their discovery established an important area of basic biology (3). PG synthesis is driven by cyclooxygenases (COX), also known as prostaglandin H₂ synthase (PGHS) or prostaglandin-endoperoxide synthase (PTGS). Cyclooxygenase was purified in 1976 from sheep and bovine seminal vesicles (4, 5). The gene was later cloned (6, 7), but the existence of a single isoform could not account for certain variable characteristics of the enzyme, including: IC₅₀ inhibitory pharmacokinetics, lags in PG synthesis, or rapid increases in PG production (8). Subsequently, these features were explained when COX-2 or PTGS-2 was cloned and found to be inducible by phorbol esters and lipopolysaccharides in human endothelial cells, monocytes, 3T3 cells and macrophages (9, 10, 84, 85). These discoveries stimulated significant interest in the development of inhibitors that were selective for each isoform, COX-1 or COX-2 (8).

Immediate-early gene expression. Key aspects of the COX-2 discovery were finding associations with inflammation and immediate-early gene expression (11). This connection led to the discovery that COX-2 was rapidly turned on in rat nontransformed epithelial cells (12). The gene exhibited typical immediate-early response characteristics. Its expression increased within 30 minutes after exposure to epidermal growth factor or tumor growth factor-α, followed by a return to baseline after 24 hours. The observation that COX-2 was upregulated by 2 to 50 fold in human colorectal adenomas and adenocarcinomas helped stimulate intense research activity to understand the association between COX-2 and cancer (13). Subsequently, COX-2 upregulation was observed in the APCmin mouse model, which harbors mutations in the adenomatous polyposis coli gene and serves as a model for familial adenomatous polyposis (14). Numerous studies later confirmed that COX-2 is consistently upregulated in a significant number of premalignant and malignant tumors (1).

The Eicosanoid pathway. Although COX molecules are central to the production of prostaglandins, numerous additional control points exist in this pathway (Fig. 1A–D; ref. 15). Upstream of cyclooxygenase lies another rate-limiting molecule, a cytosolic isoform of phospholipase A₂, which is the predominant enzyme that initiates the calcium-dependent release of arachidonic acid (AA) from the sn-2 position of membrane phospholipids.
Once released, COX enzymes convert the free AA substrate to the precursor molecule prostaglandin H2 (PGH2), which is then acted upon by various synthase molecules to generate the different PGs (Fig. 1B; ref. 8). These synthase molecules include: PGDS, PGES, PGFS, PGIS, and TXAS, which are identified by the letter of their respective PG isomer product, D2, E2, F2α, prostacyclin (PGI2), and thromboxane A2 (TXA2). The PGES subclass is heavily involved in inflammation and carcinogenesis, primarily owing to the activity of a cytosolic PGE synthase (cPGES) and two membrane-bound PGE synthases, mPGES-1 and mPGES-2 (17–19). Although multiple isoforms of PGES exist, it is mPGES-1 that is primarily responsible for...
increasing the PGE\(_2\) levels during inflammation and tumorigenesis (20).

Once the various PGs are synthesized they are exported into the extracellular microenvironment by specific multidrug resistance-associated proteins (MRP; refs. 21, 22). MRPs are expressed in virtually all tissues and cell types and facilitate ATP-dependent unidirectional transport of lipids and other organic anionic molecules. The transport of eicosanoids by the MRP/ABCC subfamily member proteins for the PGs is primarily by MRP4/ABCC4 (Fig. 1C; refs. 21, 22).

After export into the external microenvironment, the various prostanoid molecules bind to the appropriate G-protein coupled receptor (GPCR; Fig. 1D; ref. 23). Similar to the designation for the PG synthases, the eicosanoid binding GPCRs are identified by the letter of their respective PG ligand, which includes two DP\(_1\), DP\(_2\), four EP\(_1\)-EP\(_4\), FP, IP, and TP plasma membrane-bound cell surface receptors (23). These GPCRs can be activated following autocrine or paracrine stimulus in the tumor microenvironment. Among these GPCRs, EP\(_2\) and EP\(_4\) are primarily responsible for mediating PGE\(_2\)-driven proinflammatory and promalignant signals downstream. In addition to the PG binding GPCRs on the cell surface, certain nuclear receptors belonging to the peroxisome proliferator activated receptor (PPAR) family exist in the cell nucleus, which bind certain PGs (24).

The catabolism of PGs involves a two-step process. In addition to passive uptake, PGs are actively taken up by a different subset of membrane transport molecules and then acted upon by catabolic enzymes (Fig. 1C). Active uptake by organic anion transporter peptides (OATP) predominantly occurs by prostaglandin transporter (PGT), a subclass of OATP molecule (25–27). Once taken up by cells, a number of PGs are enzymatically catabolized and inactivated by NAD\(^+\)-dependent 15-hydroxyprostaglandin dehydrogenase (Fig. 1C; refs. 28–30).

**COX-2: A key molecular target.** Although much work is underway to identify targets downstream of COX-2, as the primary rate-limiting factor in this pathway it remains the key target (15). An essential aspect of COX-2 biology is its sufficiency as a single molecule to cause cancer formation in numerous transgenic mouse models (31–33). In contrast, COX-1 exhibited basal expression levels associated with COX activity. For example, endogenously produced endocannabinoids and free fatty acids were discovered to be metabolic substrates of COX enzymes (36). These new-found enzymatic functions occurred more efficiently through COX-2 activity than COX-1 (36). In the case of one endocannabinoid, anandamide metabolism by COX-2 was involved in producing D-type prostanoids that caused the death of certain tumorigenic keratinocytes (37). In a different set of functional enzyme studies, recent evidence has shown that fatty acid-mediated cross-talk occurs between monomers of cyclooxygenase homodimers, which is allosterically regulated (38). These new findings suggest that the distribution, function, and regulation of both COX-1 and COX-2 are much more complex than first believed.

**Clinical-Translational Advances**

**NSAIDs and COXIBs.** It is becoming increasingly clear that COX-1 and COX-2 regulation is very complex and experts a diverse impact on biology and physiology. A variety of side effects have now been associated with long-term use of nonsteroidal anti-inflammatory drugs (NSAID) and COX-2 selective inhibitors (COXIB) that likely reflect on-target effects (39). Prolonged use of nonselective NSAIDs, such as aspirin, ibuprofen, and naproxen, is associated with COX-1-specific side effects such as the induction of gastrointestinal complications and the promotion of bleeding through the inhibition of platelet activation. The development of COXIBs helped to alleviate these complications by selectively eliminating COX-2 activity while sparing COX-1-mediated biological effects. The COXIB drugs include: celecoxib, rofecoxib, valdecoxib, parecoxib, and etoricoxib. The degree of gastrointestinal benefit versus cardiovascular toxicity that results from chronic COXIB use seems to associate with the degree of specificity for COX-2 (39, 40).

**NSAIDs/COXIBs and cancer.** Extensive information from population studies and clinical trials indicates that regular intake of various NSAIDs reduces the risk of cancer (1) in multiple organ sites (41–46), and several randomized
trials showed significant benefit from the use of aspirin. In contrast to COX-2 selective inhibitors that act competitively, aspirin is nonselective and irreversibly inactivates both COX-1 and COX-2 (36). Regular aspirin use significantly reduces the incidence of various cancers (47). For example, prospective cohort studies involving 82,911 women enrolled in the Nurses’ Health Study (48), or 47,363 male health professionals (49), showed that regular long-term use of aspirin was associated with a significantly reduced incidence of colorectal cancer. In prospective studies that examined the recurrence of adenomatous polyps in patients with a history of resected colon cancer, daily use of aspirin was associated with a significant reduction in the incidence of colorectal adenomas (50). Another prospective study compared low dose (81 mg once daily) and high dose (325 mg once daily) aspirin with placebo to reduce adenoma formation (51). In this study, the most benefit was observed in the low-dose aspirin group (38% incidence in adenoma formation), compared with the placebo group (47%) and the high dose aspirin group (45%). In multiple breast cancer trials, regular aspirin use was also associated with a reduction in the incidence of cancer (52, 53). Multiple studies have also shown a potential reduction in lung cancer risk in association with regular aspirin use (54, 55). The benefits of aspirin use in cancer prevention have led to a recent international consensus statement to highlight the advantages (56) and the initiation of a trial focused on preventing esophageal cancer that combines aspirin with esomeprazole (a proton pump inhibitor) AspECT, to limit any gastric side effects (57).

Although the cancer preventive properties of aspirin remain encouraging, a number of case-control studies support the use of COXIBs in a prevention setting as being more selective with fewer gastrointestinal side effects. For example, the Adenoma Prevention with Celecoxib (APC) trial clearly showed the efficacy of celecoxib in preventing formation of adenomatous polyps, a precursor of colon cancer (58). The effects on advanced adenoma formation were particularly significant, 21.3% incidence in patients taking placebo, 12.5% incidence \( (P < 0.0001) \) in patients taking low dose celecoxib (200 mg twice daily), and 15.8% \( (P < 0.0001) \) in patients taking high-dose celecoxib (400 mg twice daily; refs. 59, 60). In follow-up studies on the APC trial, patients with variants in the cytochrome P450 2C9 (CYP2C9) gene exhibited impaired metabolism of celecoxib. Impaired metabolism, associated with two variants in particular, CYP2C9*2 (R144C) and CYP2C9*3 (I359L), influenced the dose-related response or toxicity of celecoxib (61). In a separate trial that employed celecoxib once daily, known as the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trial (62), the treatment group showed significantly reduced occurrence of colorectal adenomas within 3 years after polypectomy (63). In yet another randomized trial using rofecoxib called Adenomatous Polyp Prevention on Vioxx (APPROVE), the discovery of increased cardiovascular risk overshadowed the findings of a significantly reduced risk of developing advanced colorectal adenomas (63–65). The associations between the use of COXIBs and a reduction in cancer risk are not limited to colon cancer. Lung cancer incidence is also reduced through the use of COXIBs (66). Further, in a prostate cancer study, COX-2 inhibitors delayed or prevented disease progression (67). Collectively, these studies illustrate the potential benefits associated with the use of COXIBs, particularly a reduction in cancer incidence in high-risk populations.

**Genetic polymorphisms in COX-2.** Genetic variation in the COX molecules may play a role in the development of cancer. In the breast, a decrease in cancer risk was observed among women who reported using aspirin (53). In contrast, in those individuals not using NSAIDs in the same study, a COX-2 polymorphism \( (rs2143416) \) was found to be significantly associated with the development of breast carcinoma (53). In another breast cancer study, the incidence of T8473C genetic polymorphisms present in the COX-2 gene influenced risk (52). In an aspirin trial of colorectal carcinoma, two single-nucleotide polymorphisms in rs5277 and rs4648310 COX-2 alleles were associated with increased adenoma recurrence (68). In basal cell carcinoma (BCC) by contrast, polymorphisms did not influence response to NSAIDs, however, individuals harboring a variant allele of COX-2, T8473C, were at 2.27 fold higher risk of developing BCC compared with wild-type (69). In the case of lung cancer, a T8473C polymorphism in COX-2 in nonsmokers was associated with a 5.75 fold higher risk of developing lung cancer compared with wild-type allele carriers (70). Collectively these studies suggest that polymorphisms in the COX-2 gene can enhance cancer risk in a variety of cancers. Because enhanced risk was typically seen in the nontreatment groups, these data suggest that aspirin potentially may help overcome the risk associated with these COX-2 polymorphisms.

**Understanding the cardiovascular risks.** Cardiovascular toxicity, typically defined as stroke, myocardial infarction, and other thromboembolic events, has emerged as an important risk factor to consider during the regular use of NSAIDs and coxibs (71). The first study to raise concern about the use of COXIBs was the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial (72). This study compared naproxen (500 mg twice daily) with rofecoxib (50 mg/day) to examine gastrointestinal safety in patients with rheumatoid arthritis. The VIGOR study showed a fourfold lower risk for myocardial infarction in patients treated with naproxen compared with those treated with rofecoxib (72). In another study known as the Celecoxib Long-Term Arthritis Safety Study (CLASS) trial, patients with either osteo- or rheumatoid arthritis were evaluated for the gastrointestinal safety of celecoxib (73). The CLASS study showed no difference in the incidence of cardiovascular events between drug regimens in patients treated with celecoxib (400 mg twice daily) versus ibuprofen (800 mg three times a day) or diclofenac (75 mg twice daily; ref. 73). The APC colon cancer prevention trial used doses of celecoxib at 200 or 400 mg twice daily versus placebo (58). This trial showed a dose-dependent increase in the risk ratio for cardiovascular events in the...
celecoxib arms compared with placebo (58). In follow up studies, the influence of celecoxib on cardiovascular events was found to be associated with preexisting atherosclerotic heart disease (60). Cardiovascular risk assessment continues to be a concern with COXIB use and will require developing accurate predictive measures that are mechanism based and take into account the overall health status of a patient.

One potential mechanism that influences the cardiovascular risk associated with COXIB use is thought to involve shifting the hemostatic balance between antithrombotic prostacyclin (PGI2) and prothrombotic thromboxane A2 (TxA2) in the circulation (71). Platelets survive for 8 to 12 days in circulation and lack the capacity for protein synthesis. Thus once they are shed into circulation, platelets must rely on existing levels of COX-1 to initiate prothrombotic-TxA2 metabolism via thromboxane synthase. In contrast, nucleated endothelial cells lining the blood vessels continually synthesize COX-2 to produce antithrombotic-PGI2, via prostacyclin synthase. Chronic use of COXIBs at high concentrations is necessary to shift the equilibrium in favor of prothrombotic-TxA2 because endothelial cells have the ability to synthesize new COX-2 and replace depleted downstream antithrombotic-PGI2. This process is greatly amplified in patients that harbor atherosclerotic disease.

Other approaches for the clinical use of COXIBs. COXIBs may also be effective in slowing the progression of established cancer when used in combination with established cancer therapies. It was recently shown that progression free survival was longer in patients with non-small cell lung cancer (NSCLC) who expressed high levels of COX-2 in their tumors when celecoxib treatment was combined with erlotinib (an epidermal growth factor inhibitor; ref. 74). The rationale for combining celecoxib with erlotinib is supported by preclinical data that showed a reduction in adenomatous polyp formation in APCmin/+ mice with both treatments (75). In another study, NSCLC patients were treated with either celecoxib or zileuton (5-lipoxygenase inhibitor) alone or in combination in addition to carboplatin and gemcitabine (76). Patients whose tumors had moderate to high COX-2 expression had longer survival when celecoxib was added to chemotheraphy compared with those whose tumors did not overexpress COX-2 (76).

Returning to the future. Since the earliest attempts at cancer prevention there have been success stories about NSAID and COXIB use (77). Accurately assessing the risk versus the benefit that accompanies any drug is key to its safe and effective use. Unfortunately, the successful determination of risk-response profiles may take years of follow up to properly evaluate (49). This might be particularly true with regard to targeting the eicosanoid pathway because of the many potential branch points that require analysis. However, the advent of modern molecular profiling methods may provide an opportunity to evaluate risk versus response pathways relatively soon. These molecular profiling approaches are likely to include evaluating polymorphisms that drive response profiles (78), as well as evaluation of cancer risk polymorphisms (79), and cardiovascular and gastrointestinal risk polymorphisms (80–83).

Remaining mindful of the need to proactively integrate risk response evaluations into clinical trials is critical. To this end, successfully developing modern molecular profiling technologies that enable rapid assessment of risk are essential. Such technologies, when combined with standard clinical risk profiles, will help us fully understand how to best use NSAIDs and COXIBs for cancer prevention and treatment by identifying patients most likely to benefit and/or excluding those individuals at highest risk of significant toxicity.

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