In addition to the well-established pathophysiological role that COX-2 plays in inflammation, recent evidence implies that this isoform may also be involved in multiple biologic events throughout the tumorigenic process. Many epidemiological studies demonstrate that nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk of a wide range of tumors. Further, COX-2 is chronically overexpressed in many premalignant, malignant, and metastatic human cancers, and levels of overexpression have been shown to significantly correlate to invasiveness, prognosis, and survival in some cancers. Pharmacological studies consistently demonstrate that COX-2 inhibitors dose-dependently inhibit tumor growth and metastasis in various relevant animal models of cancer. Importantly, several investigators have also shown COX-2 inhibitors may act additively or synergistically with currently used cytotoxics and molecularly targeted agents. Here we present a broad overview of the growing evidence that COX-2 plays a pivotal role throughout oncogenesis and summarize the rationale to explore the use of COX-2 inhibitors for the prevention and/or treatment of cancer as a single agent or in combination with current anticancer modalities.

Introduction

The cyclooxygenases are responsible for the conversion of arachidonic acid to prostaglandins (PGs), and their metabolites play a pivotal role in multiple physiologic and pathophysiologic processes. Cyclooxygenase-1 (COX-1) is constitutively expressed in most tissues and is responsible for maintaining physiologic processes such as gastric and renal protection and platelet function. In contrast, cyclooxygenase-2 (COX-2) is induced in response to growth factors \(^1,2\) (i.e., endothelial growth factor [EGF], vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF-2]), cytokines (e.g., tumor necrosis factor-\(\alpha\) [TNF-\(\alpha\)], interleukin-\(\alpha\) [IL-\(\alpha\)], and interleukin-1\(\beta\) [IL-1\(\beta\)]), and tumor promoters (e.g., v-src, v-Ha-ras, HER-2/neu, and Wnt). \(^3,4\) COX-2 is expressed in macrophages, synoviocytes, and endothelial cells in response to inflammation and cellular activation. \(^5,7\) Conventional NSAIDs
inhibit both COX-1 and COX-2, hence they also disrupt COX-1 dependent homeostatic functions. Therefore, molecular-based targeting strategies were employed to develop specific COX-2 inhibitors to circumvent the gastric and renal toxicities caused by mixed COX inhibitors.8-10

In addition to the well-studied role of COX-2 in acute inflammatory processes, recent work clearly suggests COX-2-derived metabolites contribute at multiple points throughout tumorigenesis, including premalignant hyperproliferation, transformation, maintenance of tumor viability, growth, invasion, and metastatic spread. Here we summarize the collective evidence that supports the potential anticancer activity of COX-2 inhibitors.

Epidemiological Evidence: COX-2 and Cancer

Epidemiological studies provided the first evidence that COX may be involved in the pathogenesis of cancer. Several reports indicate NSAIDs can prevent the development of various human tumors, including colon,11-18 breast,19-22 lung,19 gastric,23,24 and esophageal24,25 neoplasias. For example, in a prospective study evaluating data from 12,668 subjects over 12.4 years, the incidence of several cancers was lower in regular users of aspirin. The incidence rate for all cancers combined was 0.83 (95% CI, 0.74-0.93), lung cancer 0.68 (95% CI, 0.49-0.94), breast cancer in women 0.70 (95% CI, 0.50-0.96), and colorectal cancer in younger men 0.35 (95% CI, 0.17-0.73).19

These data collectively provide the rationale for additional clinical trials to stringently evaluate NSAID efficacy in other types of cancers as well.

COX-2 Expression in Human Tumors

To understand the potential importance and role of COX-2 in human cancers, we developed and utilized immunohistochemical techniques to stringently characterize COX-2 in progressing stages of tumorigenesis.

Fig 1. — COX-2 is up-regulated in various solid tumors. Fig 1a shows up-regulation in head and neck, bladder, renal cell, breast, pancreatic, and prostate cancer. Fig 1b shows up-regulation in cholangiocarcinoma, hepatocellular (HCC), mesothelioma, endometrial, basal cell, and gastric cancers. Formalin-fixed, paraffin-embedded human cancers from archival tissues were prepared by immunohistology using standard methods and stained for COX-2 with isoform-specific antisera. The red stain depicts the presence of COX-2.
in human epithelial and solid tumors. COX-2 was consistently overexpressed in premalignant lesions such as oral leukoplakia, actinic keratosis, prostatic intraepithelial neoplasia, and carcinoma in situ of the bladder and breast.\textsuperscript{11,22,26} COX-2 was also up-regulated in head and neck, bladder, lung, hepatocellular, pancreatic, mesothelioma, cholangiocarcinoma, gastric, cutaneous, Kaposi’s sarcoma, and cervical cancers (Fig 1).

In general, COX-2 is up-regulated throughout the tumorigenic process, from early hyperplasia to metastatic disease (Fig 2). Elevated levels of COX-2 immunoreactivity were primarily detected in the neoplastic epithelium, inflammatory cells, and vasculature within and adjacent to tumor nests (Fig 3).

COX-2 was also consistently and more intensely observed in metastatic lesions compared with the corresponding primary tumor. In general, COX-2 is expressed in 40% to 80% of neoplastic cells in human cancers and the extent and intensity of expression is greater in cancerous than in noncancer cells. Moreover, well- and moderately-differentiated cancers have significantly higher COX-2 expression than poorly differentiated cancers. COX-2 is also detected in noncancerous cells immediately adjacent to tumor cells (<2 mm) and in the angiogenic vasculature within tumors and in pre-existing blood vessels adjacent to tumors.\textsuperscript{6} In contrast, COX-2 is not detected in the vasculature of normal tissues.\textsuperscript{27}

**Fig 2a.** — COX-2 is expressed throughout mammary carcinogenesis. As shown here, COX-2 is not detected in normal mammary epithelial cells (normal) but is clearly expressed in ductal carcinoma in situ (DCIS), ductal carcinoma, and infiltrating carcinoma. Formalin-fixed, paraffin-embedded human cancers from archival tissues were prepared by immunohistology using standard methods and stained for COX-2 with isoform-specific antisera. The red stain depicts the presence of COX-2.

**Fig 2b.** — COX-2 is up-regulated in colonic tumorigenesis. COX-2 is not detected in normal cells. However, enhanced levels of COX-2 are observed in early colonic hyperplasia (sporadic adenomatous carcinoma), primary colon cancer, and metastatic disease. Formalin-fixed, paraffin-embedded human cancers from archival tissues were prepared by immunohistology using standard methods and stained for COX-2 with isoform-specific antisera. The red stain depicts the presence of COX-2.

**Fig 3.** — COX-2 immunoreactivity in human bladder cancer. Moderate-to-strong COX-2 immunoreactivity is detected in (a) the neoplastic epithelium (CA), (b) inflammatory (macrophage) cells, and (c) vasculature endothelial cells (EC) within and adjacent to tumor nests. Tissue was excised from patients with bladder cancer and stained for COX-2 with isoform-specific antisera. The red stain depicts the presence of COX-2.
Our expression studies are consistent with those reported by others\textsuperscript{28-38} and collectively imply COX-2 activity may be responsible for increased prostaglandin levels in cancer tissues.\textsuperscript{39,40} Importantly, recent work demonstrates a relationship between overexpression of COX-2 and the invasiveness and survival of patients with breast,\textsuperscript{41} colon,\textsuperscript{42-44} gastric,\textsuperscript{45,46} and lung\textsuperscript{47} cancers.

Association Between HER-2/neu and COX-2 Expression

HER-2/neu, also known as c-erbB2, is a receptor protein kinase whose overexpression is widely accepted as an adverse prognostic marker.\textsuperscript{48} Interestingly, COX-2 is detected in a subset of hormonally driven tumors such as breast, prostate, and ovarian cancers.\textsuperscript{6} For example, COX-2 overexpression in mammary tumors appears to be correlated with HER-2/neu. In a landmark study, Subbaramaiyah et al\textsuperscript{49} evaluated COX-2 expression in 29 patients with breast cancer and reported high levels of COX-2 in 14 of 15 HER-2/neu positive patients. In contrast, only 4 of 14 HER-2/neu negative patients expressed COX-2, and detected levels were much lower than in any of the HER-2/neu positive patients. Recently we observed that COX-2 and HER-2/neu are similarly distributed in breast cancer tissue and are often coexpressed in hyperproliferating, dysplastic, and neoplastic epithelial cells.\textsuperscript{50} In another study, we evaluated archival sections of human mammary lesions, including DCIS, ductal, lobular, infiltrating, and mucinous mammary cancers for HER-2/neu and COX-2. Of the 25 cases evaluated, 15 were HER-2/neu positive and 10 were HER-2/neu negative. Both COX-2 and HER-2/neu were overexpressed in 3 of 3 cases of DCIS and 10 of 12 cases with ductal, lobular, and infiltrating carcinomas, and both were over expressed in the same cells. COX-2 was expressed less often in HER-2/neu negative breast cancers.\textsuperscript{51}

To determine if these two oncogenes were functionally linked, HER-2/neu and COX-2 negative breast cancer cells were engineered to express HER-2/neu. COX-2 was strongly induced in HER-2/neu transfected cells but was not expressed in null transfected cells.\textsuperscript{52} Collectively, these data suggest that HER-2/neu may modulate the overexpression of COX-2 in human cancer.\textsuperscript{52} However, the upstream modulator(s) of COX-2 in ovarian and prostate cancer have yet to be elucidated.

The correlation between COX-2 and HER-2/neu expression in human breast cancers may have important treatment implications. For example, patients with early disease such as DCIS are often given the option of treatment vs no treatment after lumpectomy. These data collectively imply HER-2/neu/COX-2 positive patients with DCIS may represent a high-risk patient population that may benefit from treatment with celecoxib. Additionally, combination therapy with celecoxib and other molecular-targeted agents such as aromatase inhibitors (ie, exemestane) or agents that block HER-2/neu activation may also prove beneficial in clinical trials of breast cancer. Indeed, future research on the effect of COX-2 inhibition on HER-2/neu positive vs HER-2/neu negative mammary tumors will significantly contribute to the present understanding of the potential clinical utility of COX-2 inhibitors for the prevention and/or treatment of human breast cancer.

Mechanisms of COX-2–Associated Tumorigenesis

COX-2 is overexpressed along the continuum of oncogenesis and is likely to be a key player in a number of biologic pathways leading to cancer. Current evidence indicates that COX-2 promotes tumor-specific angiogenesis,\textsuperscript{53-56} inhibits apoptosis,\textsuperscript{57,60} and induces proangiogenic factors such as VEGF,\textsuperscript{61,62} inducible nitrogen oxide synthetase promoter (iNOS),\textsuperscript{63} IL-6,\textsuperscript{64} IL-8,\textsuperscript{65} and TIE-2.\textsuperscript{66}

COX-2-derived metabolites from infiltrating inflammatory cells undoubtedly contribute to the tumorigenic process as well. For example, enhanced prostaglandin synthesis may contribute to oncogenesis by directly stimulating mitogenesis in fibroblasts,\textsuperscript{67} osteoblasts,\textsuperscript{68,69} and mammary epithelial cells.\textsuperscript{70} Excessive local synthesis of prostaglandins has also been shown to disrupt immune surveillance that may otherwise suppress tumor growth.\textsuperscript{71,72} In addition, the direct product of COX-2, PGH\textsubscript{2}, can isomerize by both enzymatic and nonenzymatic reactions to form the potent mutagen malondialdehyde, which can induce frame shifts and base pair substitutions.\textsuperscript{73} Additional free radical damage may occur via the peroxidative activity of COX-2, which can efficiently oxidize aromatic and heterocyclic amines and dihydrodiol derivatives.\textsuperscript{74}

Increased prostaglandin levels may be particularly important during the progression of breast cancer. PGE\textsubscript{2} has recently been shown to stimulate aromatase transcription,\textsuperscript{75,76} leading to supraphysiologic local estrogen levels, which in turn leads to the subsequent release of growth factors and enhanced proliferation.\textsuperscript{77} Immunohistochemical analysis by Brueggemeier et al\textsuperscript{78} supports the association between COX-2 and aromatase. The cytochrome P450 enzyme aromatase (CYP19) and COX-2 were coexpressed in all cases of human breast cancer, and a significant linear association was found between levels of CYP19 gene expression and COX-2 gene expression (R - 0.80, P<.0001).\textsuperscript{78}

In addition to increasing aromatase transcription, COX-2-induced PGE\textsubscript{2} also promotes angiogenesis,\textsuperscript{53,55}
which is required for tumor growth and metastasis.\(^{79,80}\) Taken together, these data suggest that both autocrine and paracrine mechanisms may be responsible for the development and/or progression of estrogen-dependent breast cancer either directly by stimulation of tumor cell proliferation or indirectly by enhanced prostaglandin-regulated local estrogen synthesis (Fig 4).

**Anticancer Activity of COX Inhibitors in Relevant Animal Models of Cancer**

Retrospective and/or epidemiological studies of conventional NSAID use and incidence of cancer led to the hypothesis that COX-derived metabolites play an important role in tumorigenesis. The inhibitory effect of COX inhibitors on tumor growth in relevant animal models of various epithelial cancers supports this hypothesis.\(^\text{81-86}\) However, maximum efficacy in these studies is typically dose-limited by COX-1-related toxicities. In contrast, COX-2 inhibitors have been shown to markedly inhibit tumor growth and metastasis in several animal models of colon,\(^\text{87-90}\) skin,\(^\text{91,92}\) lung,\(^6\) bladder,\(^93\) and breast cancers.\(^\text{94,95}\)

For example, sporadic colorectal adenomas and adenocarcinomas can be generated by administering azoxymethane (AOM). When tested in this model, celecoxib effectively prevents the development of colon tumors in 93% of rats and inhibits tumor initiation by approximately 80%.\(^\text{88,96}\) In this model, the chemopreventive effect of celecoxib was dose-related and treatment was effective during both initiation and postinitiation phases of the disease.\(^\text{97}\)

Likewise, Harris et al\(^\text{94}\) evaluated the chemopreventive potential of celecoxib and ibuprofen in the 7,12-dimethyl-benz(a)anthracene (DMBA) model of breast cancer in female Sprague-Dawley rats and compared the results with untreated control animals. All rats were pretreated 7 days before DMBA administration and therapy was continued for 105 days. Malignant tumors developed in 100% of control animals; 95% had multiple tumors and tumor size was >1.5 cm\(^3\). Both celecoxib and ibuprofen significantly suppressed the incidence, burden, and volume of malignant tumors and prolonged the latency period of tumor induction compared with controls. However, the effects of celecoxib were significantly more pronounced than those of ibuprofen (Table).\(^\text{94}\)

The chemotherapeutic activity of celecoxib was next evaluated on established DMBA-induced mammary tumors by Alshafie and coworkers.\(^\text{95}\) DMBA-treated rats were randomized to control or celecoxib following 6 weeks of DMBA treatment. Mammary tumors continued to grow in the control group, with an average increase in tumor size of 500% vs baseline. In the celecoxib-treated group, tumor regression was reported in approximately 90% of animals, and tumor was reduced 32% compared with baseline (\(P<0.04\)). In contrast, 10 new mammary tumors were identified in the control group (30% increase). The number of palpable tumors decreased in celecoxib-treated animals from 24 to 18 (25%; \(P<0.05\)); no decrease in the number of tumors was observed in the control group.\(^\text{95}\)

Chemopreventive Effects of Celecoxib and Ibuprofen: Mammary Cancer Incidence, Tumor Burden, Tumor Volume, and Latency of Tumor Induction in a DMBA Model of Breast Cancer

<table>
<thead>
<tr>
<th>Effect vs Control</th>
<th>Celecoxib</th>
<th>Ibuprofen</th>
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</thead>
<tbody>
<tr>
<td>Reduction in tumor incidence, %</td>
<td>68*</td>
<td>40*</td>
</tr>
<tr>
<td>Reduction in tumor burden, %</td>
<td>86*</td>
<td>52*</td>
</tr>
<tr>
<td>Reduction in tumor volume, %</td>
<td>81*</td>
<td>57*</td>
</tr>
<tr>
<td>Latency period of tumor induction, days(^3)</td>
<td>95</td>
<td>86</td>
</tr>
</tbody>
</table>

* \(P<0.001\) vs controls
† \(P<0.01\) vs ibuprofen
‡ 58 days in controls
Data from Harris et al.\(^\text{94}\)
cantly reduced tumor incidence and growth (P=.001), with the effect being maximal when celecoxib was fed during both the initiation and promotion phases.98

Based on the association between COX-2 and aromatase gene expression,75,78 Pesenti and colleagues99 evaluated the therapeutic potential of celecoxib (500 ppm in diet) and exemestane (50 mg/kg weekly) alone and in combination in the DMBA model of breast cancer. Female Sprague-Dawley rats with at least one tumor measuring 1 cm in size were studied. An objective response (OR) rate of 48% was achieved with combination therapy, compared with OR rates of 5% in rats treated with exemestane alone and 0 in rats treated with celecoxib alone and in control rats. The development of new tumors followed a similar pattern. These data demonstrate that the combination of exemestane and celecoxib is more effective than either agent alone in reducing tumor burden and volume, as well as the incidence of new tumors in this animal model.99 Moreover, it demonstrates that, while celecoxib inhibits the exaggerated induction of aromatase, it does not eliminate baseline or physiologic aromatase production. The addition of exemestane is required, therefore, to block the activity of aromatase enzyme whose production is not blocked by celecoxib.

Antiangiogenic agents have also been shown to enhance the antitumor activity of cytotoxic drugs when given in combination in rodent models of cancer.100,101 In addition to anti-inflammatory properties, recent work convincingly demonstrates that celecoxib can also act as a potent antiangiogenic agent.54 We therefore evaluated the antitumor activity of celecoxib as a monotherapy and in combination with cytotoxic chemotherapy in the HT-29 human colon carcinoma xenograft model and in the Lewis lung carcinoma syngeneic model. The combined modality of celecoxib with cytotoxic agents was more effective in both cancer models than either agent alone. Recent work also suggests that COX-2 inhibitors may potentiate radiation therapy by increasing cellular radiosensitivity and greatly enhancing tumor response when these two modalities are administered in combination.102,103

Discussion and Conclusions

We have presented data to support the hypothesis that COX-2 activity modulates critical steps in the initiation, promotion, and progression of several human epithelial cancers. COX-2-derived prostaglandins may promote the development of cancer by various mechanisms, including stimulation of tumor cell growth and neovascularization.5360,75,78,104-106 Thus, it is not surprising that COX-2 inhibitors such as celecoxib markedly inhibit tumor growth and metastasis in a dose-dependent manner in numerous animal models of solid tumors.87,90,94 However, the exact manner by which celecoxib reduces tumorigenesis has not been fully elucidated.

The ultimate goal of cancer treatment is to specifically prevent the growth of precancerous or cancerous cells without affecting normal cells. This is particularly important in chemoprevention and treatment of early disease, which typically involves long-term treatment to healthy subjects. Therefore, NSAID selection should be based on safety as well as efficacy. Recent research has clearly established that specific COX-2 inhibitors are associated with less toxicity than the conventional mixed COX inhibitors.10

In summary, COX-2 is overexpressed in both early and late stages of carcinogenesis and has been shown to be efficacious as monotherapy and in combination with conventional chemotherapeutics in relevant animal models. Taken together, the epidemiologic data and preclinical studies in animal models have generated compelling interest in the potential use of COX-2 inhibitors in chemoprevention and chemotherapy of human tumors. Clinical trials will be necessary to determine whether COX-2 inhibitors will provide clinical benefit, as well as to define the intervention points during tumor progression that will allow for optimal efficacy.

References


