Cyclooxygenase-2 in Gastrointestinal Malignancies

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Cyclooxygenase (COX) is an enzyme complex that plays an important role in the conversion of arachidonic acid to prostaglandins. Prostaglandins are essential modulators of signal transduction pathways, which contribute to the metastatic properties of gastrointestinal (GI) malignancies. Although COX-1 is constitutively active, COX-2 is upregulated by cytokines, growth factors, and mitogen. COX-2 is involved in malignant cell proliferation, angiogenesis, migration, invasion, and antiapoptotic activity. Thus, COX-2 inhibitors may represent a promising therapeutic strategy for the treatment of GI cancers. In this review, the role of COX-2 in GI cancers is explored, and its clinical applications as a therapeutic target are discussed.

KEYWORDS: cyclooxygenase, gastrointestinal malignancies, metastasis, prostaglandins, target.

INTRODUCTION

Cyclooxygenase (COX) is a key enzyme that is essential for prostaglandin (PG) synthesis. PGs regulate signal transduction pathways and also are involved in cell-to-cell adhesion, development, and differentiation. In vivo, PGs have both autocrine and paracrine properties on tumor cell proliferation and migration. COX-2 has 2 distinct isoforms with diverse properties and biologic functions. COX-1 is expressed in various human tissues and cellular organs, whereas COX-2 is specifically expressed as a proinflammatory enzyme and becomes active only in response to certain stimuli like cytokines, mitogens, and growth factors. COX-2 expression is significant in gastrointestinal (GI) cancer progression because it stimulates tumor proliferation, inhibits apoptosis, induces angiogenesis and metastasis, and participates in tumor invasion and immunosuppression (Fig. 1).1-3

Many investigations have revealed the association of overexpressed COX-2 in GI malignancies, such as esophageal, liver, pancreatic, colorectal, and gastric cancers. COX-2 expression increases many proangiogenic factors, namely, fibroblast growth factor, hepatocyte growth factor, and vascular endothelial growth factor (VEGF).4,5 In GI malignancies, COX-2 is significantly associated with microvessel density (Fig. 1).6 In vivo studies reveal that PGs derived from COX-2 aid in activating angiogenesis, and inhibition of COX-2 reduces neovascularization.7 Retrospective cohort studies have revealed a strong relation between COX-2 overexpression and patient survival rates.8 Many investigations are currently evaluating the efficiency of selective COX-2 inhibitors that could serve as potential anticancer agents, either alone or in combination with other cancer therapies like chemotherapy and radiotherapy.9,10 These combination treatments incorporating COX-2 inhibitors with chemotherapeutic agents could serve in the future as potential strategies in the treatment of GI malignancies.

COX-2 INHIBITION IN ESOPHAGEAL CANCER

Esophageal cancer has an estimated incidence of approximately 572,034 cases and 508,585 fatalities in 2018.11 Esophageal cancer is the eighth most frequent cancer and the sixth main cause of cancer-associated deaths around the world. COX-2 is a crucial gene for regulating cell growth, variation, and apoptosis in many cancer types. COX-2 overexpression is highly associated with adverse clinicopathologic features of esophageal squamous cell carcinoma (ESCC).12 COX-2 is an independent feature for prognosis in patients with ESCC and thus could be used as a promising target factor for ESCC therapies.

Another study by Shao et al13 revealed that microRNAs (miRNAs) aid in regulating expression levels of COX-2 in ESCC by suppressing cell proliferation, migration, and invasion. The overexpression of miR-101 in ESCC reduces proliferation, angiogenesis, invasion, and migration (Fig. 2).13 COX-2 also was targeted by miR-101, which confirms the
antimetastatic activity of miRNAs on COX-2 expression levels. Therefore, miRNAs also could serve as potential targets in ESCC treatments.

A natural compound, Rhizoma paridis saponins (RPS), can inhibit the growth of ESCC in both in vitro and in vivo tumor models. RPS considerably suppressed tumor development in an in vivo esophageal cancer model. It also decreased N-nitrosomethylbenzylamine-mediated COX-2 overexpression and cyclin D1 expression levels in esophageal tissues (Fig. 2). RPS promoted apoptosis and cell-cycle arrest and inhibited COX-2 signaling. These results suggest that RPS may be a novel target for antitumor treatments. An investigation was conducted to study zinc transporter 5 (ZIP5) expression levels in esophageal human tissues and cancer cell lines. ZIP5 expression was at its peak in ESCC, intermediary in paracarcinoma, and lowest in normal tissue samples. Knockdown of ZIP5 expression levels reduced the proliferation and metastasis of esophageal cancer cell lines and suppressed cyclin D1 and COX-2 levels (Fig. 2). Wu et al. established that another zinc transporter, ZIP6, has a key function in the diagnosis of ESCC, with greater ZIP6 expression levels correlating to a shorter survival interval in patients with metastatic ESCC. ZIP6 knockdown repressed the growth and metastasis of ESCC cell lines.

COX-2 INHIBITION IN GASTRIC CANCER

COX-2 is usually upregulated in gastric cancer cell lines and precursor lesions. As a valuable prognostic factor, it provides important clinical information. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been promoted extensively for preventing gastric cancer. To reduce GI toxicity, however, selective COX-2 antagonists have been developed and used as chemopreventive drugs for gastric cancer treatment. However, selective COX-2 inhibitors (COXBIs) are correlated with severe cardiovascular events. To reverse such toxic effects of NSAIDs and COXBIs, the combination of acid-suppressing drugs along with NSAIDs could serve as a breakthrough in gastric cancer treatment. It has been revealed that, through the inhibition of COX-2, COXBIs can inhibit the advancement of gastric cancer.

In gastric cancer, COX-2 is highly expressed in the epithelium of cancer and dysplastic glands. It is also overexpressed in the gastric mucosa of Helicobacter pylori-infected patients and in H. pylori-induced gastric adenocarcinoma. Recent investigations have revealed that COX-2 expression is reduced significantly after H. pylori eradication in patients with gastric cancer. Numerous studies have revealed that COX-2 expression is even higher in the initial stage of gastric cancer and progressively increases with the worsening of lesions in the gastric mucosa. Previous studies also have demonstrated that COX-2 expression is elevated in patients with gastric cancer. These results together reveal that COX-2 expression is elicited by H. pylori infections and is a primary occurrence during tumorigenesis in the stomach. In addition, COX-2 upregulation improves the probability of both invasion and metastasis and is associated with a poor prognosis in patients who have gastric cancer.

COX INHIBITION IN PANCREATIC CANCER

Pancreatic cancer (PC) represents 1 of the most aggressive human tumors. Very few chemopreventive agents have been successful in acting against PC cell lines. One of the targeted therapies for the prognosis and prevention of PC is COX through its 2 isoforms. COX genes, COX-1 and COX-2, are rate-limiting steps during PG synthesis. The role of COX genes in the advancement of pancreatitis also is well known. Most studies have demonstrated upregulated levels of COX in PC at both the messenger and protein levels. One study revealed that a lack of COX-2 developed minimum pancreatitis in vivo, whereas a lack of COX-1 expression developed severe pancreatitis. Epigenetic silencing of COX-1 is very common in PC. It is a well-established finding that COX-2 is responsible for producing pancreatic duct cells to produce PGs. COX-1 expression is lacking in pancreatic cells, which explains why aspirin, a primary
COX-1 inhibitor, is not responsible for preventing the progression of PC.\textsuperscript{31}

Several reports have suggested that COX-2 is upregulated in PC and is implicated in cell-cycle progression and metastasis, including angiogenesis and invasion.\textsuperscript{32-35} COX-2 messenger RNA can be stabilized by V-Ki-Ras\textsuperscript{2} Kirsten rat sarcoma 2 (k-ras) mutations in PC.\textsuperscript{36} Furthermore, COX-2 inhibitors can suppress cell proliferation, induce apoptosis, and enhance sensitivity to chemotherapy in PC.\textsuperscript{33,37} Preclinical studies using the selective COX-2 inhibitor celecoxib demonstrated that enhanced the growth suppressive activity of the small-molecule inhibitor erlotinib in PC cell lines by downregulating epidermal growth factor receptor (EGFR), COX-2, and human epidermal growth factor receptor 2 (HER2).\textsuperscript{38} In addition, the combination decreased the activity of nuclear factor κ B (NF-κB), thus further contributing to EGFR and COX-2 downregulation and potentiating apoptotic pathways.\textsuperscript{38} These findings suggest that combined targeting of the COX-2 and EGFR pathways in PC might be a potential strategy for PC treatment and prevention.

Moreover, clinical studies have demonstrated that celecoxib displays an enhanced safety profile compared with other nonselective COX antagonists in the context of renal and GI toxicity.\textsuperscript{39} Unfortunately, celecoxib did not improve the cytotoxic activity of gemcitabine and cisplatin in PC clinical investigations. These disappointing results do not dismiss the potential significance of COX-2 as a therapeutic target in PC and suggest that the careful selection of patient subpopulations who are responsive to COX-2 inhibitors and combination regimens should be the focus of future clinical trials.

**COX-2 INHIBITION IN HEPATOCELLULAR CARCINOMA**

Hepatocellular carcinoma (HCC) is also the third most frequent cause of tumor-related deaths worldwide.\textsuperscript{40} Li et al\textsuperscript{41} evaluated the effects of meloxicam on HCC cell proliferation and migration. The results of their study revealed that meloxicam could inhibit the proliferation and cell-cycle arrest of HCC cells. It also suppressed the ability of HCC cells that overexpressed COX-2 and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) to migrate by elevating expression levels of E-cadherin and decreasing the expression of matrix metalloproteinase 2 (MMP-2) and MMP-9 (Fig. 3). It is known that COX-2 and PGE\textsubscript{2} stimulate the β-catenin signaling that promotes tumor migration (Fig. 3). It was demonstrated that the nuclear accumulation of β-catenin was enhanced considerably upon treatment with PGE\textsubscript{2}, and meloxicam also could reverse the stimulation of glycogen synthase kinase 3 β in HCC cell lines.\textsuperscript{41} Therefore, these outcomes offer a potential therapeutic approach targeted against HCC growth and proliferation.

Another study suggested that, after patients underwent transcatheter arterial chemoembolization (TACE), the levels of hypoxia-inducible factor 1α (HIF-1α) and COX-2 proteins in HCC cells were considerably higher...
compared with the levels in adjacent normal tissues as well as the levels in tissues from patients with HCC who did not undergo TACE. Patients who underwent TACE and had increased levels of HIF-1α and COX-2 had a very short survival compared with that of patients who had negative levels of HIF-1α and COX-2. This indicates that the detection of both HIF-1α and COX-2 may be useful for evaluating patients with HCC who undergo TACE. Moreover, after TACE, the prognosis of patients with HCC was associated with: 1) tumor size, 2) intrahepatic metastasis, and 3) COX-2 expression, which are known as independent predictive factors in individuals with HCC. HIF-1α expression was increased in the hypoxic environment produced by TACE, which, in turn, upregulated COX-2 expression to induce angiogenesis, inhibit apoptosis, enhance tumor invasion, and then enhance migration. Higher expression of HIF-1α stimulates epithelial-mesenchymal transition alterations, which increase cell migration and then invasion. It is known that TACE triggers many inflammatory reactions and is the most significant indicator of a poor prognosis in patients with HCC.

Elevated levels of COX-2 expression also have been observed in human HCC cell lines; and increased levels of PGs, particularly PGE2, have been reported in HCC cell lines. COX-2 overexpression and HCC treatment with exogenous PGE2 have been associated with the induction of tumor development and invasion. It is well known that COX-2 inhibitors (NSAIDs) inhibit tumor proliferation and induce apoptosis in cultured HCC cell lines. Well differentiated HCC cell lines tend to have elevated levels of COX-2 expression compared with the levels in less differentiated tumor tissues. Preserved COX-2 expression has been demonstrated in dysplastic nodules and in the early stages of HCC development. Those investigations indicate that COX-2 could be involved in the initial stages of liver tumorigenesis. Increased COX-2 expression levels have been associated with induced VEGF levels, microvessel density, and tumor invasion in HCC cell lines. This finding suggests that COX-2 may play a role in promoting HCC by modulating angiogenesis. Elevated EGFR signaling as a result of overexpression, alteration, or autocrine signaling loops also has been associated with HCC. The relation between PG signaling derived by COX-2 and other growth-regulatory signaling pathways, such as the EGFR, c-Met, and VEGF, could be important for developing future anti-HCC combinational therapy.

**COX INHIBITION IN COLORECTAL CANCER**

The human prostaglandin-endoperoxide synthase 1 (Ptgs1) gene (COX-1) has a length of 22 kb, 11 exons, and many transcriptional start sites that are present on chromosome 9. The COX-1 promoter is rich in GC

![Diagram of COX-2 inhibition](image-url)
content because it lacks CAAT or TATA box signals. These properties are distinctive to “housekeeping” genes.53 Thromboxane A2 (TXA2) and PGE2 are prostanoids or lipid mediators and are obtained from arachidonic acid through activation of the COX-1 gene (Fig. 4). These prostanoids also are responsible for homeostatic functions (Fig. 4).54,55 High levels of lipid mediators are released through the activation of platelets upon interaction with colorectal cancer (CRC) cell lines.56 Platelets are implicated in the early stages of tumorigenesis and metastasis.57 COX-1 expression is induced in both platelets and gastric epithelial cell lines, in which it also fulfills a key role in initiating the activation of platelets by generating TXA2.58 COX-1 initiates gastric cytoprotection by generating PGE2 to induce mucosal protection.58 Traditionally, platelet function is affected by the irreversible blocking of platelet COX-1 action and even by repressing the adenosine diphosphate receptor P2Y12.59 Recent studies have revealed that active COX-1 initiates the signal for COX-2 expression in activated platelets.60,61 The antiplatelet agents aspirin and thienopyridines were unable to suppress COX-2 levels in the nucleus.62 This explains how the activity of COX-1 in activated platelets serves as an inducing signal for overexpression of the COX-2 gene (Fig. 4). Recent investigations have revealed that inactivation of COX-1 might inhibit COX-2 upregulation in adjacent cells of the innermost layer of the GI tract at the site of CRC.63 Conversely, however, COX-2 has been identified as a primary response gene but is also expressed in many malignant tissues.64 COX-2 is typically induced as a response to cytokines, hormones, tumor promoters, growth factors, and stress (Fig. 4).52 Both COX-1 and COX-2 synthesize the exact same product, prostaglandin H2 (PGH2), which is transformed later into prostanoids by various synthases. A study involving patients with stage III CRC concluded that the use of aspirin and a COX-2 inhibitor was strongly correlated with decreased mortality and recurrence.65 Outcomes from ongoing trials (the Aspirin in Dukes C and High-Risk Dukes B Colorectal Cancers[ASCOLT] study and Cancer and Leukemia Group B study CALGB 80702) are awaited that could provide further directionality in this field.65 Additional investigations of aspirin and COX-2 inhibitors as predictive biomarkers will be essential for future analyses. The use of EGFR or EGFR inhibitors plus COX-2 inhibitors could provide an effective way to inhibit the carcinogenic properties of COX-2 overexpression.67 Because of the important role played by EGFR in CRC progression and metastasis, monoclonal antibodies like cetuximab and panitumumab have been successfully developed as neutralizing antibodies.66-68 A phase 2 investigation evaluating the combination of capecitabine, irinotecan, and celecoxib in CRC demonstrated that the

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**Figure 4.** The role of cyclooxygenases (COXs) in colorectal cancer (CRC) growth and metastasis is illustrated. Cytokines and growth factors induce COX-2 production from arachidonic acid and COX-1. COX-1 is involved in the synthesis of thromboxane A2 (TXA2) and prostaglandin E2 (PGE2) and leads to homeostasis. COX-1 and COX-2 are involved in prostaglandin H2 (PGH2) and PGE2 synthesis, which binds to lymphatic cells to induce CRC metastasis. In addition, PGE2 also induces inflammation and tumor growth.
COX-2 inhibitor celecoxib did not enhance the antitumor action of capecitabine and irinotecan. These findings indicate that the development of COX-2 blockade markers aimed at identifying patients who could benefit from the addition of celecoxib to chemotherapy will be required before future clinical investigations.

Marsoni et al. recently revealed that the combination of trastuzumab plus lapatinib produced positive outcomes in patients who were pretreated for HER2-positive, metastatic CRC. The binding of HER3 and HER4 to the growth factor heregulin leads to the dimerization with HER2, the upregulation of COX-2 production, and the elevation in CRC cell growth. The functionality of the amplified expression of COX-2 is demonstrated by the huge accumulation of PGE2 as a result of COX-2 activation. The significance of increased PGE2 in CRC growth is evident from the subsequent expression of motility-related phenotypes. Increased expression of PGE2 is linked to effects on the cytoskeleton and cell-to-cell junction remodeling, which distinctly amplifies cell motility.

CONCLUSION AND FUTURE PROSPECTS
COX-2 plays a significant role in GI tumor development and metastasis by stimulating cell proliferation, inhibiting apoptosis, and inducing new vessel formation and immunosuppression. COX-2 expression is upregulated in GI cancers and provides valuable clinical information as a prognostic factor. COX-2 overexpression is also associated with the NF-kB pathway and H. pylori infection. The COX-2/PGE2 signaling pathway helps in tumor growth through the creation of an inflammatory microenvironment. Consequently, targeting the COX-2/PGE2 and HIF-1α/NF-κB signaling pathways is a therapeutic option for GI malignancies.

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