Introduction

Since epidemiological studies associated a decreased incidence of colorectal cancer with the long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), several lines of evidence have been accumulated that cyclooxygenase (COX) which is a molecular target of NSAIDs and prostaglandins (PGs) derived by COX are concerned in carcinogenesis. PGs are hormone-like bioactive substances mediating autocrine and paracrine signaling over short distances and are involved in many physiological and pathological processes. Levels of COX-2 and COX-2 derived PGE2 are increased in several human premalignant and malignant tumors including colorectal cancers and adenomas. Studies in experimental models of colon carcinogenesis show that selective COX-1 or COX-2 inhibitors reduce tumor formation and growth. Clinical studies have been initiated to determine the chemoprotective effects of selective COX-2 inhibitors in patients with familial adenomatous polyposis or sporadic adenoma. Possible cardiovascular effects will need to be taken into account in an assessment of the potential ability of any of these drugs to prevent neoplasia in the large bowel and other organs. Based on the fact that PGE2, involved in almost all events concerning carcinogenesis, further examinations concerning mPGES-1, EP receptors, and 15-PGDH, should be promoted as a target for colon cancer prevention and therapeutics. Hence, detailed analyses are needed to clarify PGE2 related carcinogenesis.

Abstract

PGE2 plays a key role in colon carcinogenesis. Evidence continues to accumulate that cyclooxygenase-2 (COX-2), an inducible COX isoform, represents a potential pharmacological target for the prevention and treatment of cancer, including tumors affecting the large bowels. Several mechanisms of COX-2 related tumor promotion have been identified. Some are dependent on PGE2 production (such as induction of cell proliferation, angiogenesis or local immunosuppression, inhibition of apoptosis, increase in cell motility). COX-2 expression has been demonstrated in epithelial cells of colorectal cancers and adenomas. Studies in experimental models of colon carcinogenesis show that selective COX-1 or COX-2 inhibitors reduce tumor formation and growth. Clinical studies have been initiated to determine the chemoprotective effects of selective COX-2 inhibitors in patients with familial adenomatous polyposis or sporadic adenoma. Possible cardiovascular effects will need to be taken into account in an assessment of the potential ability of any of these drugs to prevent neoplasia in the large bowel and other organs. Based on the fact that PGE2, involved in almost all events concerning carcinogenesis, further examinations concerning mPGES-1, EP receptors, and 15-PGDH, should be promoted as a target for colon cancer prevention and therapeutics. Hence, detailed analyses are needed to clarify PGE2 related carcinogenesis.

Keywords cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1 (mPGES-1), colorectal cancer, chemoprevention, COX-2 inhibitor

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PGs and thromboxanes formed by COX-catalyzed oxidation of arachidonate depend, therefore, on the exact composition of different synthases in different types of cells. Additionally, each of the products derived from PGH₂ has its own range of biological activities.

There are at least two isoforms of COX (COX-1 and COX-2 that have significant differences in the gene and promoter structures). The gene for COX-1 is on human chromosomes 9 and that for COX-2 is on human chromosomes 1. COX-1 is expressed in most tissues and appears to be responsible production of PGs that mediate normal physiological functions, such as maintenance of the integrity of the gastric mucosa and regulation of renal blood flow. In contrast, COX-2 is undetectable in most normal tissues. It is induced by cytokines, growth factors, oncogenes, and tumor promoters, and therefore contributes to the synthesis of PGs in inflamed and neoplastic tissues. The 5'-flanking region of the COX-2 gene contains numerous cis-acting promoter elements, including NF-κB, NF-IL-6, PEA3 and CRE sites that are important for mediating gene expression. The mRNA of COX-2 is unstable compared with COX-1 mRNA, because it contains 17 copies of the Shaw-Kamen sequence (AUUUA) in the 3'-untranslated region of COX-2. These instability sequences are typical of immediate, early response genes. Also, the 5'-flanking region of COX-2 contains a TATA motif. This is missing in the gene for COX-1, which is typical for a housekeeping gene.

Selective inhibition of COX-2 is achievable despite normal physiological functions, such as maintenance of the integrity of the gastric mucosa and regulation of renal blood flow. In contrast, COX-2 is undetectable in most normal tissues. It is induced by cytokines, growth factors, oncogenes, and tumor promoters, and therefore contributes to the synthesis of PGs in inflamed and neoplastic tissues. The 5'-flanking region of the COX-2 gene contains numerous cis-acting promoter elements, including NF-κB, NF-IL-6, PEA3 and CRE sites that are important for mediating gene expression (Fig. 2). The mRNA of COX-2 is unstable compared with COX-1 mRNA, because it contains 17 copies of the Shaw-Kamen sequence (AUUUA) in the 3'-untranslated region of COX-2. These instability sequences are typical of immediate, early response genes. Also, the 5'-flanking region of COX-2 contains a TATA motif. This is missing in the gene for COX-1, which is typical for a housekeeping gene.
the similar structures of the active sites of COX-1 and COX-2 proteins. This is so because a single substitution of isoleucine in COX-1 with valine in the NSAID binding site of COX-2 creates a larger active site with a void volume to the side of the central active site channel in COX-2. Compounds designed to bind in this additional space are potent and selective inhibitors of COX-2.

(2) Evidence that COX-2 contributes to colon carcinogenesis

Expression of COX-2 in colon tumor

The production of PGs, especially PGE \(_2\), rises in large bowel cancer. \(\text{PGF}_2\) plays a key role in colon carcinogenesis because overproduction of PGE \(_2\) is found in tumor tissues of familial adenomatous polyposis. Several analyses of cell culture and animal models make sure the importance of PGE \(_2\) in carcinogenesis. \(\text{PGH}_2\) derived from arachidonic acid by COX-1 or COX-2 is converted into PGE \(_2\) by tissue specific PGE synthase. Among the kinds of PGE synthase that are already discovered, analyses of mPGES-1, cPGES but by mPGES-1 that is also induced at the same time in tumor tissue. Overexpression of both COX-2 and mPGES-1 is observed in colon, lung, head and neck, breast, stomach, uterus and penis cancer. In addition, expression of COX-2 and mPGES-1 is detected in the intestinal polyp stromal fibroblasts of APC\(^{+/−}\) mice. This suggested that PGE \(_2\) is produced efficiently on the occasion of polyp formation.

(3) Mechanisms of COX-2 related cancer biology

Inhibition of Apoptosis

The size of the cell population of the gastrointestinal tract depends on the balance between cell proliferation and cell death via apoptosis. Decreased apoptosis is observed in premalignant and malignant neoplasms of the colon. The regulation of apoptosis is cell-type-specific, and depends on the balance between positive and negative effectors. Of the many factors that regulate apoptosis, there is an inverse relationship between Bcl-2 and apoptosis. Rat intestinal epithelial cells, stably overexpressing COX-2, express increased levels of Bcl-2, and are resistant to undergo apoptosis when stimulated by butyrate. Addition of PGE \(_2\) to human colon cancer cells has similar effects. Treatment with the NSAID sulindac sulfide reversed the resistance to apoptosis induced by overexpression of COX-2. Taken together, these data show a clear causal linkage between the expression of COX-2 and the inhibition of apoptosis. It is possible that upregulation of COX-2 prolongs the survival of abnormal cells, and thereby favors the accumulation of sequential genetic changes that increase the risk of tumorigenesis.

Angiogenesis

Since tumor growth depends on a corresponding increase in blood supply, tumor cells secrete vascular growth factors, e.g., vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) that stimulate angiogenesis. Overexpression of COX-2 in colon cancer cells enhanced the production of VEGF, the migration of endothelial cells and the formation of tubular networks. Also, another COX-2 expressed colon cell constitutively stimulated angiogenesis in co-cultures with endothelial cells. These effects were blocked by a selective inhibitor of COX-2.

Invasiveness

One of the key events in the evolution of metastatic cancer is the ability of an aberrant cell to invade locally and spread to distant sites. When human colon cancer cells were permanently transfected with a COX-2 expression vector, production of PGs increased, and the cells became more invasive as compared with control or parental cells. Increased invasiveness was associated both with activation of membrane metalloproteinase-2 and in-
creased mRNA expression of the membrane-type metalloproteinase-1. These enzymes digest the collagen matrix of the basement membrane stimulating the invasive and motile phenotype of tumor cells. Both the increased production of PGs and invasiveness were reversed by treatment with sulindac sulfide(51).

Immunosuppression

The growth of tumors typically is enhanced by immunosuppression(52). Colony-stimulating factors released by tumor cells activate monocytes and macrophages to synthesize PGE₂, which inhibits the production of immune regulatory lymphokines, T- and B-cell proliferation, and the cytotoxic activity of natural killer cells. PGE₂ also inhibits the production of tumor necrosis factor while including the production of interleukin-10, which has immunosuppressive effects. Moreover, PGE₂ has profound effects on the production of cytokines by T cells leading to the enhancement of Th helper 2 (Th2) type response. By contrast, PGs inhibit the production of Th1 cytokines(53). Recent studies have shown that the Th1/2 balance is shifted toward a Th2 immune response by malignancy(54). Thus, the overproduction of COX-2 derived PGs could result in the inhibition of cell-mediated antitumor response.

(4) Anti-tumor effect of COX-2 inhibitor

Inhibitory effect of carcinogenesis

When APC-2716 mice, a murine model of FAP, were crossed with mice containing targeted mutations that inactivate Pgst2 gene, the size and number of small intestinal and colonic polyps decreased in comparison with the Pgst2 wild type strain(55). Also when APC-2716 mice, another FAP model, were treated with sulindac, intestinal polyp formation was decreased(56). As already mentioned, since expression of COX-2 and mPGES-1 is detected in the intestinal polyp stromal fibroblasts of APC-2716 mice, PGE₂ is produced efficiently on the occasion of polyp formation(57). Moreover, PGE₂ produced by COX-2 and mPGES-1 stimulates angiogenesis through signal transduction by EP2 receptor, and activates EGF receptor which plays an important role of the tumor growth(56, 58). Mechanisms of contribution to anti-tumor effects by COX-2 inhibitor have been shown by the inhibition of these phenomena by COX-2 inhibitor. On the other hand, anti-tumor effects of COX-2 inhibitor have also been reported to be independent of COX-2 inhibition(56-67), and the effects of COX-2 inhibitor appear diverse.

Anti-metastatic effect

Several experiments were reported to prove that COX-2 inhibitor could inhibit metastasizing to the liver. The numbers of the metastatic nodules were decreased by the treatment with JTE-522, NS-398, rofecoxib, or etodolac as a COX-2 inhibitor compared with control(68-77).

Various mechanisms were revealed of these inhibitory effects of liver metastasis. NS-398 can reduce cell proliferation associated with decreased expression of cyclin D1(71) and invasiveness by inhibition of both MMP-2 and MMP-9 protein levels and activity(70). JTE-522 can suppress angiogenesis via VEGF expression on HT29 cells(70). JTE-522 also can suppress invasiveness by reducing MMP-2 secretion. Etodolac suppresses liver metastasis by reducing MMP-9 activity(71). 

(5) Clinical trial in FAP and sporadic adenoma patients treated with COX-2 inhibitor to prevent colon cancer

Several randomized, double-blind, placebo-controlled clinical trials have investigated the effects of the NSAID sulindac on adenoma risk in patients with familial adenomatous polyposis (FAP). Steinbach studied the effect of celecoxib on colorectal polyps in patients with FAP. Seventy-seven patients were treated with celecoxib (100 or 400 mg twice daily) or placebo for six months. After six months, the patients receiving 400 mg of celecoxib twice a day had a 28.0% reduction in the mean number of colorectal polyps and a 30.7% reduction in the polyp burden(72). Higuchi studied the effect of 9 month’s treatment with rofecoxib on colorectal polyps in FAP(73). Twenty one patients were assigned, the polyp number and size in the rofecoxib group decreased as compared with the placebo group. Treatment with rofecoxib did not significantly increase any adverse events. However, the polyp size and number increased after the end of treatment. This suggests that long-time administration would be needed to maintain the effects of COX-2 inhibitor(80). Additionally, some sulindac resistant-patients or resistant-adenomas in FAP were also reported(81-84). Therefore, the positioning of the treatment with COX-2 inhibitor in patients with FAP is considered not to replace prophylactic total colectomy, but to delay the timing of surgical treatment or to prevent against remnant rectum.

Recently, two reports of large-scale clinical trials concerning COX-2 inhibitors and sporadic colorectal adenomatous polyps demonstrate that COX-2 inhibitor reduced the occurrence of colorectal adenomas within three years after polypectomy(85-87). However, adverse cardiovascular events were increased by celecoxib or rofecoxib treatment. COX-2 inhibitors cannot be recommended routinely.

(6) Current problems and future prospects

As mentioned above, as there are several lines of evidence that COX-2 is concerned with carcinogenesis, growth, invasion and metastasis of large bowel cancer, the treatment with COX-2 inhibitor has been considered to have clinical benefit for cancer prevention and anti-metastatic effect. Although the toxicities of COX-2 inhibitor did not increase as compared with non-selective
NSAIDS in CLASS trial\(^{88}\) and Vioxx trial\(^{89}\), meta-analysis revealed that COX-2 inhibitors can increase the incidence of cardiovascular events compared with aspirin. Possible cardiovascular effects will need to be taken into account in an assessment of the potential ability of any of these drugs to prevent neoplasia in the large bowel and other organs\(^{90}\). Recently, SNPs in COX-1 and CYP2C9 are reported to be apparently associated with elements of drug response by coxibs\(^{91}\). Additionally, Cheng et al. reported that deletion of mPGES-1 depressed PGE\(_2\) expression, augmented PG\(_I_2\) expression, and had no effect on thrombogenesis or blood pressure. This suggests that inhibitors of mPGES-1 may retain their anti-inflammatory effect by depressing PGE\(_2\), while avoiding the adverse cardiovascular consequences associated with COX-2 mediated PG\(_I_2\) suppression\(^{92}\).

Recently, both transcript and protein of 15-PGDH which enzymatically degrade PGE\(_2\), have been reported to be highly expressed in normal colonic epithelia but not in colon cancers. It is also demonstrated that 15-PGDH has tumor suppressor activity for colon cancer\(^{93-95}\). 15-PGDH plays a role for the maintenance and accumulation of PGE\(_2\) in colon cancer tissue.

Based on the fact that PGE\(_2\) is involved in almost all events concerning carcinogenesis\(^{96}\), further examinations concerning mPGES-1 that overexpresses in cancer tissue, and EP receptors\(^{97-100}\), the receptor for PGE\(_2\) which connect to proliferation signals, and 15-PGDH as tumor suppressor, should be promoted as a target for colon cancer prevention and therapeutics (Fig. 4). COX-1 has been pointed out the relation to carcinogenesis with which assumed not to concerned conventionally\(^{97, 98, 101}\). Hence, it should not be simple COX-2 related preventive and therapeutic models which had been built; detailed analyses must be needed to clarify PGE\(_2\) related carcinogenesis.

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References

![Fig. 4 Possible other targets related PGE\(_2\). Candidate targets are surrounded by a yellow oval. ➧: blocking of target molecule should be needed. ➤: up-regulation should be needed.](image-url)


