Cyclooxygenase Isoforms in Tumorigenesis

**Abstract**

Cyclooxygenases (COXs) are the key enzymes in prostanoid biosynthetic pathway. Prostanoids act as local hormones and they are involved in numerous normal physiological processes but also in the development of various diseases. COXs are present in three isoforms: COX1, COX2 and COX3. COX1 is usually involved in maintaining the physiological conditions of the healthy tissues while COX2 is mainly related with various malignancies. COX3 is a functional splice variant of COX1 and its role is still controversial.

COX1 has a housekeeping role while COX2 has been implicated in tumorigenesis. COX2 overexpression has been detected in premalignant and malignant lesions in different tissues. Numerous experiments confirmed its role in all stages of tumor development. It was reported that COX2 participates in promotion of angiogenesis and inhibition of apoptosis and immunosurveillance. The exact mechanisms of its activity in these processes are not fully clarified. Expression of COX2 has been proposed to be an early event in colorectal tumorigenesis.

Different drugs were developed to target COX and they were effective in preventing cancer but they also exhibited adverse effects. There is extensive research conducted in order to develop the drug that wouldn’t cause severe toxicities and to define the polymorphisms that affect the patients response to a certain drug but also to define the cancer risk polymorphisms.

**INTRODUCTION**

Cyclooxygenases (COXs), also known as prostaglandin H synthases (PGHSs), are enzymes that catalyze the key regulatory step in prostanoid biosynthetic pathway.

Prostanoids are biologically active lipids that include prostaglandins, thromboxanes and prostacyclins. They act as local hormones in both autocrine and paracrine manner binding to their cell surface receptors on the target cells. Prostanoids are involved in a multitude of normal physiological processes. Variety of these processes includes gastrointestinal mucosa protection, platelet aggregation, maintenance of vascular homeostasis, different aspects of kidney function and reproduction. Prostanoids are also implicated in various pathophysiological responses. Production of prostanoids is elevated in inflammation in which they play an important role. Other pathological conditions include cancer and some other diseases (1–3).

Cyclooxygenases are the rate limiting enzymes in prostanoid synthesis. They act in two enzymatic conversions: a cyclooxygenase reaction in which arachidonic acid is converted to prostaglandin G2 (PGG2) and
a peroxidase reaction in which PGG$_2$ is reduced to prostaglandin H$_2$ (PGH$_2$). PGH$_2$ is then converted to various prostanoids by specific synthases (Figure 1) (4–6).

### CYCLOOXYGENASE ISOFORMS

The great progress in understanding prostaglandin biosynthesis was made in 1964 when arachidonic acid was identified as prostaglandin precursor (4, 8). The enzyme involved in the conversion of arachidonic acid to prostaglandin was at that time referred to as prostaglandin synthetase and through series of experiments the precise role of this enzyme, today known as COX (or PGHS) was clarified (4, 6, 9–13).

Since the early 1970s scientists suspected that variants of COX enzyme might exist (14). Experiments in late 1980s and early 1990s led to the confirmation that there is more than one isoform of COX (6, 11, 15, 16). Hla and Neilson (17) published the sequence of the second isoform of the enzyme and named it COX2. Experimental results suggested that it has a role in inflammatory process (17, 18).

The question of the existence of an additional COX isoform was raised while investigating effects of anti-inflammatory drugs on PG synthesis in experimental animals (19). Soon, the third variant named COX3 was described (20).

### Cyclooxygenase 1 and Cyclooxygenase 2

COX1 and COX2 are protein products of two different genes (Figure 2A). COX1 is encoded by a gene located on the chromosome 9 (21) and contains 11 exons (22). COX2 gene is located on chromosome 1 (23) and contains 10 exons. In comparison to COX1 it lacks an exon that encodes the putative signal peptide (24). COX1 and COX2 proteins were found to be approximately 600 amino acids in size (Figure 2B) (5, 6). They share in average 60% of amino acid sequence identity and also show high similarity in tertiary and quaternary structure. COXs are homodimers. In each monomer three domains can be distinguished: an epidermal growth factor domain, membrane binding domain and catalytic domain at C-terminus (25–27).

Although COX1 and COX2 show high degree of homology and have the same role in the PG biosynthetic pathway, the structure of their active site (27), kinetics (28) and catalytic mechanisms (29) somewhat differ. There are also subtle differences in subcellular compartmentation, differential expression and specific interactions with terminal synthases in prostanoid synthesis. COX1 shows greater structural stability of the active sites and overall structural stability than COX2. These differences contribute to the distinct functions that are assigned to COX1 and COX2 isoenzymes (5, 30).

COX1 has been generally referred to as constitutively expressed isoform and COX2 as an inducible isoform.

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**Figure 1.** The role of COX in biosynthesis of prostanoids. COX catalyzes conversion of arachidonic acid to PGH$_2$, which is then further converted to different prostanoids by the activity of specific synthases and isomerases.
COX1 was reported to be expressed in normal tissues where it was involved in regulating physiological processes and maintaining physiological conditions. It is involved in platelet aggregation, parturition, maintenance of the gastric mucosa (31). COX2 on the other hand was reported to be undetectable in normal tissues but implicated in pathological processes. Closer investigating of the COX2 expression in normal tissues gave more complex results (32–35). COX2 is also involved in physiological processes like ovulation, blastocyst implantation and neonatal development (31). It was also implicated in the gastric mucosal defense together with COX1 (36). COX1 and COX 2 are also both constitutively expressed in kidneys where they act as modulators of physiological functions (3,37).

COX in tumorigenesis

COX3 is the third cyclooxygenase enzyme reported. It is in fact a splice variant of COX1 gene with retained intron 1 (20). COX3 shares the important structural and catalytic properties with COX1 and COX2. The intron 1 retention could alter folding of the protein and may affect dimerization and the active site (20). Qin and coworkers (38) confirmed the existence of this COX1 splice variant mRNAs that contained additional 94 bp at the 5’ end in comparison to COX1 sequence as a result of intron 1 retention and protein variants in different human tissues.

COX3 is interesting for further investigating because it shows sensitivity to analgesic/antipyretic drugs that have low antiinflammatory activity. It may have a role in pain and fever processes (20).

Cyclooxygenase 3

Cyclooxygenase 3

Figure 2. A The structure of COX1 and COX2 genes. B Structural differences between COX protein isoforms.

CYCLOOXYGENASES IN TUMORIGENESIS

Inflammation is the first pathological condition that COX was implicated in (39). COX2 has a very important role in initiation and resolution of inflammation. It was thought to be responsible only for production of proinflammatory factors but it has been discovered that some COX2-derived metabolites possess antiinflammatory properties (40). Till today, COX was implicated in various pathologies including cancer. Extensive experimental evidence strongly associate inflammation with tumorigenesis. For example, nonsteroidal antiinflammatory drugs (NSAIDs) were shown to significantly reduce the risk of developing breast, lung, prostate and colorectal cancer when regularly administered (41). Although the precise cause-effect sequence is not fully understood, it is well known that chronic inflammation provides tumor-promoting microenvironment (42) and it was associated with all stages of tumor progression (43). Inflammation results in higher production of variety of cytokines (including IL1, TNFα) and transcription factors (including NFκB and STAT3) that are known to upregulate COX2 (44). Generally, COX2 expression is considered to be elevated in inflammatory conditions by proinflammatory mediators and growth factors, while COX1 is considered to be constitutively expressed and to produce physiologically essential prostanoids. COX1 promoter is found to be characteristic for «housekeeping» genes and COX2 gene has multiple transcription factor binding sites in its regulatory region (for example for CRE–cAMP response element, IL6, NFκB) that indicates a complex pattern of gene regulation (45).
Prostaglandins were reported to be elevated in gastrointestinal cancer tissue in comparison to surrounding normal tissue (46). Prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) were linked to promotion of tumorigenesis (47–49). PGE2 was confirmed as a procarcinogenic agent in experiments on animal models. In mice lacking PGE2 receptors EP1, EP2 or EP4, inhibition of tumorigenesis was noticed (5). COX2, through PGE2 synthesis, plays a role in different stages of tumorigenesis: initiation, development and progression. Experiments in mouse mammary epithelium showed that COX2 overexpression itself may induce tumorigenesis (50). Investigation on skin cancer (51) and mouse intestine epithelium (52) showed that COX2 expression is not sufficient for tumor initiation but that only facilitates it. Oshima and coworkers (53) provided direct genetic evidence that COX2 plays a role in colorectal tumorigenesis. Both inactivation of COX2 gene and inhibition of COX2 by selective inhibitors reduced the polyp number and size in APC<sup>D716</sup> mice. In breast cancer, HER2/neu gene amplification is an example of the genetic alteration that is involved in its initiation and progression. Subbaramiah and coworkers (54) reported COX2 and HER expression to be significantly correlated in breast cancer. The role of COX2 in invasion of cancer cells was also investigated. COX2 was reported to be essential for epithelial-mesenchimal transition induced by TGFβ (55). Tsujii and coworkers (56) reported that COX2-expressing colon cancer cells produced higher levels of matrix metalloproteinase-2 protein and membrane-type matrix metalloproteinase-1 RNA which explained the higher extracellular matrix degrading activity noted in comparison to the same cell line lacking COX2. PGE2-mediated cancer cell migration and invasion were reported to involve PI3K/Akt pathway activation via transactivation of epithelial growth factor receptor by Src. Buchanan and coworkers (57) found that expression of epidermal growth factor receptor correlates with COX2 in colorectal cancer. PI3K/Akt pathway is considered to be involved in all aspects of COX2-mediated neoplastic progression (48, 57, 58).

COX2 has been reported to affect different processes that drive tumorigenesis including angiogenesis, inhibition of apoptosis and inhibition of immunosurveillance (Figure 3) (59). The exact way that COX2 promotes angiogenesis is not fully elucidated. Wang and coworkers (60) reported that PGE2 induces growth-regulated oncogene α (CXCL1) expression and that CXCL1 mRNA and protein expression level correlate with PGE2 levels in colorectal cancer. CXCL1 is a chemokine previously reported as a potent mediator of tumor-associated angiogenesis (61, 62). The correlation between COX2 and VEGF expression has been detected (63). Another mechanism through which COX2 is involved in tumorigenesis is inhibition of apoptosis. Overexpression of COX2 in tumor cells has been shown to affect Bcl-2. Members of Bcl-2 family are regulators of apoptosis. It is believed that COX2 changes the ratio of proapoptotic and pro-survival Bcl-2 proteins. It was reported that COX2 also modulates immune response. PGE2 negatively regulates T and B cell proliferation, cytotoxicity (by decreasing activity of natural killer cells), and cytokine production (IL-12 and tumor necrosis factor) (63).

Genetic variations may induce disregulation of gene expression or amino acid sequence modification. Either way, the normal activity of the protein product can be affected. Although most polymorphisms have no effect, some polymorphisms in COX2 were reported to be associated with cancer development. For example, SNP rs2143416 was found to be significantly associated with the risk of

![Figure 3. The role of COX2-derived PGE2 in tumorigenesis. PGE2 binds to EP receptor and stimulates tumor progression by activating different signaling pathways.](image-url)
the development of breast carcinoma (64). SNP T8473C (rs5275) was shown to influence the risk of both lung cancer (65) and breast cancer (66) while basal cell carcinoma risk was associated with polymorphisms T8473C (rs5275) and A-1195G (rs689466) (67). A promoter polymorphism A-1195G (rs689466) was also associated with an increased risk of digestive system cancers (68). These and many other association studies gave insight into the alterations at the genetic level that might affect the tumorigenesis in humans. Defining SNPs associated with different cancers and determining their functional significance, would not only enable assessing the risk of an individual to develop certain type of cancer but it might also enable personalized approach to the therapy of the disease.

Overexpression of COX2 is generally considered to be involved in tumorigenesis. COX2 can be overexpressed due to disbalanced transcription regulation or due to genetic alterations inside COX2 gene. Activated ras and TGFβ were reported to increase COX2 expression mostly by stabilizing COX2 mRNA (69, 70). However, subsets of cancer exist in which COX2 is not overexpressed so there might exist specific mechanisms for its downregulation. Downregulation by the activity of tumor suppressors is one potential mechanism. Subbaramaiah and coworkers (71) reported COX2 to be susceptible to wild-type p53 suppression and thus suggested that classical genetic changes can interact with COX2 expression. Another example of tumor suppressor that downregulates COX2 is APC (72). In colorectal cancers, aberrant methylation of COX2 was detected and the methylation closest to the transcription start site was associated with silencing of the gene (73). Low COX2 expression reported in some subsets of tumors might not necessarily mean low production of procarcinogenic prostanooids. Tumors with low COX2 expression may have upregulated prostanooids biosynthesis through other enzymes like COX1 (74).

COX1 is considered to have a "housekeeping" role mediating production of prostanooids involved in maintainance of normal physiological conditions (11, 31). COX1 activity can be inhibited by antiinflammatory drugs. In patients consuming NSAIDs for a longer period of time, severe adverse effects evolved. This was due to inhibition of COX1 which resulted in disruption of biosynthesis of protective COX1-derived prostanooids (75).

It was reported that COX1-derived prostanooids play a role in the initial phase of acute inflammation (3). Unlike COX2, there has been no connection between COX1 and the development of chronic inflammation or tumors (44). Different polymorphisms were reported in COX1 gene and it was shown that in in vitro systems several polymorphisms alter the basal COX1 metabolism (76). Genetic alteration in COX1 gene might affect COX1 expression or function and therefore impact the production of beneficial prostanooids. If this is the case, the tissues with attenuated COX1-mediated protection might be more susceptible to malignant changes. For example, Arisawa and coworkers (77) found a significant association of the promoter polymorphism C-1676T (rs1330344) and the development of gastric ulcer. And the L15_L16del polymorphism was associated with increased risk of colorectal adenomas (78). Apart from few previously mentioned studies that found correlation between COX1 polymorphisms and pathological alterations of the tissues, there has been no firm evidence for the involvement of COX1 in tumorigenesis.

CYCLOOXYGENASE IN COLORECTAL CANCER TUMORIGENESIS

COX isozymes are detectable in normal tissues of the gastrointestinal tract. As it was previously described, COX1 has a protective role in the gastrointestinal mucosa of animal models and humans. No significant alterations have been detected in COX1 expression in the colorectal malignant transformation. COX1 levels in cancer were found the same or slightly decreased in comparison to the surrounding normal tissue (79, 80). COX2 was also detected as a constitutive isoform in certain cell types in the mouse and human colon (74, 81), but its role in the intestine pathogenesis remains to be of particular interest. COX2 was first associated with colorectal cancer, and cancer in general, by Eberhart and coworkers (82) who detected elevated COX2 levels in about 80% adenocarcinomas and 40% adenomas. Experiments on mouse models confirmed this finding and proved that it plays an important role in colorectal tumorigenesis (53, 83). Numerous studies on human colorectal cancer reported COX2 overexpression when compared with normal adjacent mucosa (59, 84–88). The elevated levels of COX2 in cancer tissues may be partially due to abnormal functioning of the COX2 promoter, which suggests that constitutive formation of COX2 is an early event in colorectal tumorigenesis (89). It was also found overexpressed in premalignant lesions which supports that finding (53, 90, 91). Sheehan and coworkers (85) reported the correlation of increased levels of COX2 with the advanced tumor stage, tumor size and positive lymph node status. It was also suggested that it may correlate with tumor recurrence (87) and shorter patients survival (85). Increased tumor size and invasion, but not the development of metastasis, were also associated with COX2 overexpression by Fujita and coworkers (79). COX2 overexpression is not uniformly distributed in the intestinal malignancies. Dimberg and coworkers (92) reported significantly higher levels of COX2 in rectal cancers than in comparison to the other cancer sites. It was also found that COX2 expression is higher in sporadic cancers than in hereditary nonpolyposis colorectal cancer (97).

Different studies investigating COX polymorphisms in human colorectal cancer were conducted. Polymorphisms may affect gene’s expression if located in the regulatory regions or the amino acid structure if located in the coding sequence. Not all detected polymorphisms were reported to influence colorectal tumorigenesis (94, 95). Lin and coworkers (96) detected seven variants in COX2 gene but only one, T1532C (rs5273), was found to be associated with colorectal neoplasia. They suggested that it
has a protective role. Some SNPs, for example, G306C (rs5277) and A8897G (rs648310) were associated with increased adenoma recurrence (97). Cox and coworkers (98) reported that carriers of the minor allele of the polymorphism A1806G (rs648298) had a significantly increased risk of colorectal cancer while Iglesias and coworkers (99) associated this polymorphism with good prognosis. Using statistical analysis, Pereira and coworkers (100) gave a systematic review based on previously published literature where they concluded that only polymorphisms G-899C (rs20417) and A-1195G (rs689466) were associated with increased risk of colorectal cancers. Discrepancies between the results obtained by different studies, like the one previously mentioned, arise from variables present in the experiments. Defining these parameters would enable a more precise outcome data. Data validation is essential in order to attain the informations that could eventually be used in the clinics.

**CYCLOOXYGENASE-BASED THERAPY**

NSAIDs, for example aspirin, inhibit prostanoid synthesis (39) and through many experiments it was detected that it can inhibit cancer development (101). Regular intake of NSAIDs can reduce the risk of developing cancer (75). When applied in low doses NSAIDs act in a COX-dependent manner, while COX-independent activity was noted when applied high doses (101). It seems that both are important although the mechanisms of action are not fully elucidated (59).

As a result of NSAIDs inhibition of COX, arachidonic acid accumulates which stimulates ceramide synthesis by activating enzyme sphingomyelinase and causes cytochrome C release by changing the mitochondrial permeability. Both of these events induce apoptosis. NSAIDs can act and COX-independently by inhibiting directly activation of NFkB or inhibiting binding of PPARδ to target DNA which also results in apoptosis. It can also induce p21 expression which enables G1 cell cycle arrest (75).

Inactivating COX, NSAIDs exhibit various adverse effects mostly of cardiovascular system and affect the integrity of gastric mucosa. This effects were assigned to the inhibition of «housekeeping» COX1, besides targeted COX2, which has an important role in maintaining homeostasis. Therefore, selective inhibitors of COX2 (COXIBs) were developed. The slight difference in size and shape of the active sites between isoforms (27) was used as the basis for the drug’s selectivity. COXIBs proved to be very effective in anti-cancer therapy. More than 200 agents that selectively inhibit COX2 have been considered but till now only celecoxib is FDA approved. In some clinical trials it was shown that COXIBs can also cause cardiovascular toxicities. Explanation for this adverse effects lies in the complex interactions between different prostaglandins, prostacyclines and thromboxanes. Changed ratio of COX2- and COX1-derived prostanoids, caused by COX2 inhibition, may induce disregulation of normal physiological processes (75).

New approach to COX2 downregulation has been tested using small interfering RNAs and microRNAs. RNA interference showed great potential in COX2 silencing in cell culture and animal models on both transcriptional and posttranscriptional levels. The method gave promising results due to its efficiency without causing severe adverse effects but there is still much research to be done (102).

In order to develop drugs that wouldn’t interfere with the biosynthesis of prostanoids that have a protective role in the tissues, the investigators turned towards other potential therapeutic targets located downstream the prostanoid biosynthetic pathway. Synthesis of PGE₂ is interesting because of its role in the development of cancer and other pathologies. Micromosal prostaglandin E₂ synthase-1 (mPGES-1) uses COX2-derived PGH₁ in order to convert it to PGE₂ and it seemed to be a promising therapeutic target. It had been extensively studied but its efficiency has not yet been validated (103).

There are still many questions in the field of COX-related pharmacology. Many experiments that strive to answer these questions are conducted at the present. Hopefully they will provide better insights in the pharmacodynamics but also in the pharmacokinetics of known drugs. Genetic polymorphisms can play a role in the activity of COX in the cell, but also in the response of an individual to a certain drug. Based on novel findings, the population of patients that would benefit from particular drugs and population of patients that are at risk to develop severe adverse effects are to be determined. On the other side, there is also the need to further clarify the impact of prostanoids and COX isoenzymes on tumorigenesis in order to find new potential approaches to cancer therapy.

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