PERIODICUM BIOLOGORUM
VOL. 112, No 4, 441–449, 2010

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Overview

Cyclooxygenase Isoforms in Tumorigenesis

TINA CATELA IVKOVIĆ

Laboratory for Personalized Medicine,
Division of Molecular Medicine, Ruđer
Bošković Institute, Bijenička c. 54
Zagreb, Croatia
E-mail: tcatela@irb.hr

Received October 9, 2010.

Abstract

Cyclooxygenases (COXs) are the key enzymes in prostanoid biosynthetic pathway. Prostanoids act as local hormones and they are involved in numerous normal physiological proceses 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, maintainance 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 cycloxygenase reaction in which arachidonic acid is converted to prostaglandin G_2 (PGG_2) and

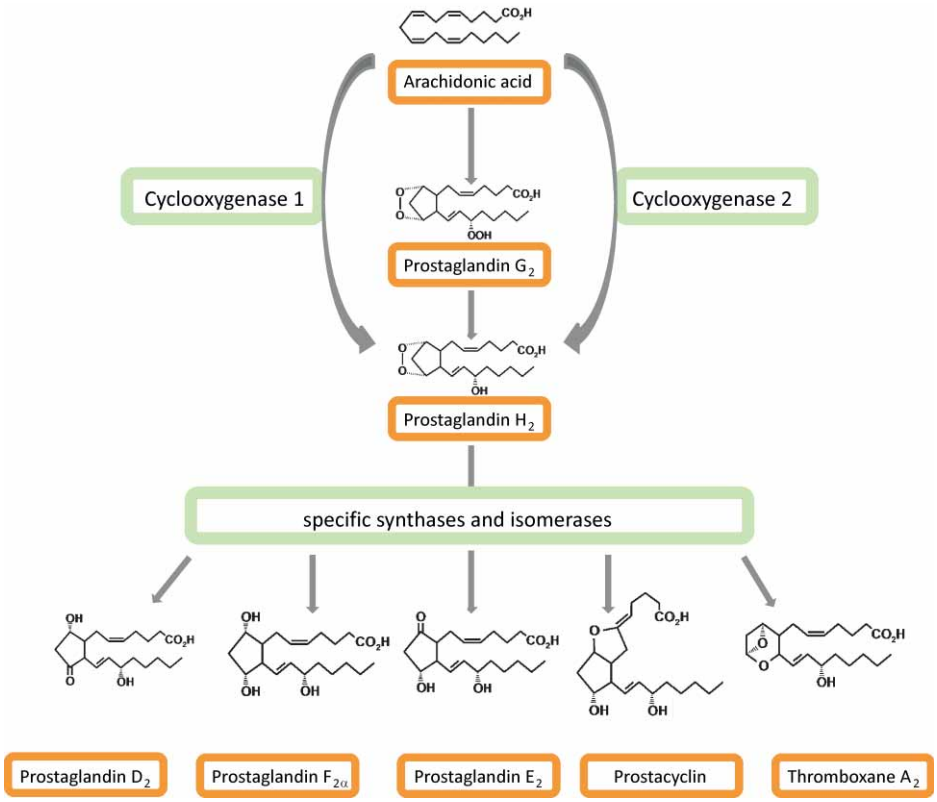

Figure 1. *The role of COX in biosynthesis of prostanoids. COX catalyzes conversion of arachidonic acid to PGH_2 which is then further converted to different prostanoids by the activity of specific synthases and isomerases.*

a peroxidase reaction in which PGG_2 is reduced to prostaglandin H_2 (PGH_2). PGH_2 is then coverted 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 reffered 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 existance 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 isoenyzmes (*5*, *30*).

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

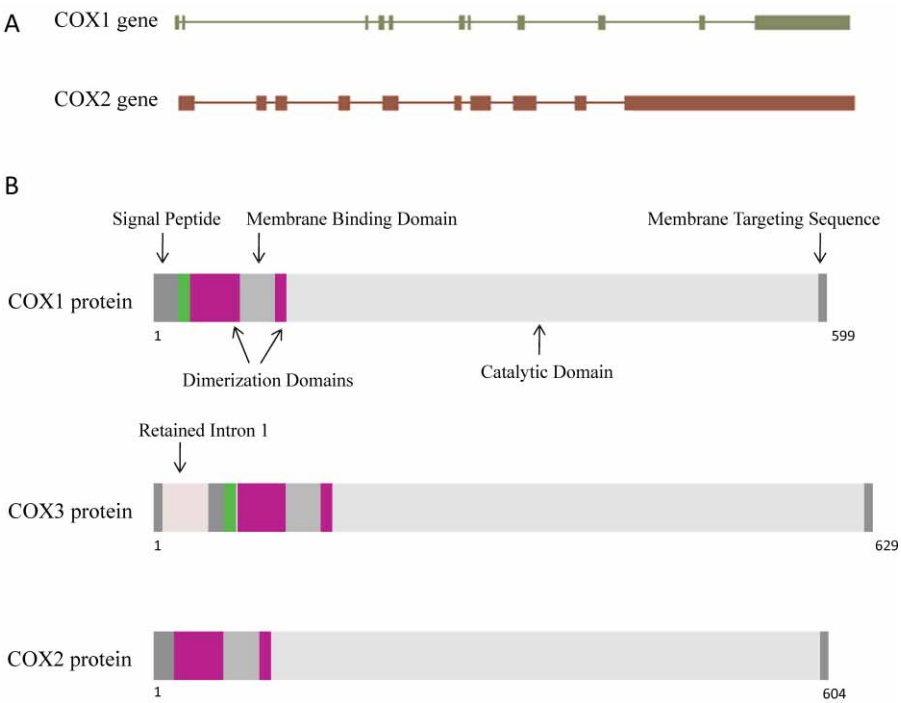

Figure 2. *A The structure of COX1 and COX2 genes. B Structural differences between COX protein isoforms.*

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, maintainance 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 physiologic functions (*3*, *37*).

Cyclooxygenase 3

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 existance 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*).

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 microenviroment (*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* promotor 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 E_2 (PGE_2) and thromboxane A_2 (TXA_2) were linked to promotion of tumorigenesis (*47–49*). PGE_2 was confirmed as a procarcinogenic agent in experiments on animal models. In mice lacking PGE_2 receptors EP1, EP2 or EP4, inhibition of tumorigenesis was noticed (*3*). COX2, through PGE_2 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 $APC^{\Delta 716}$ mice. In breast cancer, HER2/neu gene amplification is an example of the genetic alteration that is involved in its initiation and progression. Subbaramaiah 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-mesenhimal 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. PGE_2-mediated cancer cell migration and invasion were reported to involve PI3K/Akt pathway activation via transactivation of epidermal 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 PGE_2 induces growth-regulated oncogene α (CXCL1) expression and that CXCL1 mRNA and protein expression level correlate with PGE_2 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 protein family are regulators of apoptosis. It is believed that COX2 changes the ratio of proapoptotic and prosurvival Bcl-2 proteins. It was reported that COX2 also modulates immune response. PGE_2 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

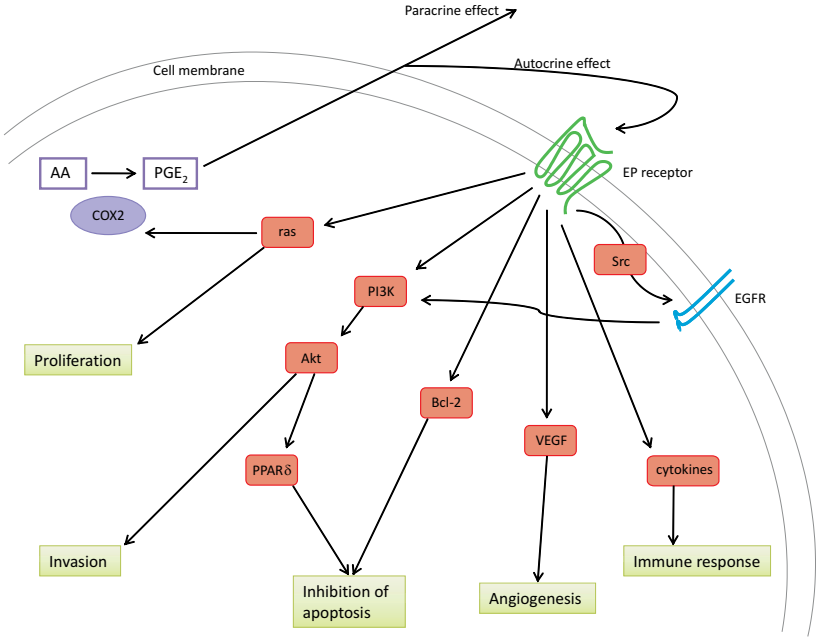

Figure 3. *The role of COX2-derived PGE_2 in tumorigenesis. PGE_2 binds to EP receptor and stimulates tumor progression by activating different signaling pathways.*

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 develope 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 prostanoids. Tumors with low COX2 expression may have upregulated prostanoids biosynthesis through other enzymes like COX1 (*74*).

COX1 is considered to have a »housekeeping« role mediating production of prostanoids involved in the 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 prostanoids (*75*).

It was reported that COX1-derived prostanoids 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 prostanoids. 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 (*34*, *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* promotor, 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 (*93*).

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 (rs4648310) were associated with increased adenoma recurrence (*97*). Cox and coworkers (*98*) reported that carriers of the minor allele of the polymorphism A1806G (rs4648298) 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 ih 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 NFκB 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 PGE_2 was interesting because of its role in the development of cancer and other pathologies. Microsomal prostaglandin E_2 synthase-1 (mPGES-1) uses COX2-derived PGH_2 in order to convert it to PGE_2 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 pharmacodinamics 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.

REFERENCES

1. COOK J A 2005 Eicosanoids. *Crit Care Med 33(12 Suppl):* S488–91
2. HARIZI H, CORCUFF J B, GUALDE N 2008 Arachidonic-acid--derived eicosanoids: roles in biology and immunopathology. *Trends Mol Med 14(10):* 461–469
3. SMYTH E M, GROSSER T, WANG M, YU Y, FITZGERALD G A 2009 Prostanoids in health and disease. *J Lipid Res 50*: S423–428
4. VAN DER OUDERAA F J, BUYTENHEK M, NUGTEREN D H, VAN DORP D A 1977 Purification and characterisation of prostaglandin endoperoxide synthetase from sheep vesicular glands. *Biochim Biophys Acta 487(2)*: 315–331
5. SMITH W L, DEWITT D L, GARAVITO R M 2000 Cyclooxygenases: Structural, cellular and molecular biology. *Annu Rev Biochem 69*: 145–182
6. SIMMONS D L, BOTTING R M, HLA T 2004 Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. *Pharmacol Rev 56(3):* 387–437
7. BERGSTRÖM S, DANIELSSON H, SAMUELSSON B 1964 The enzymic formation of prostaglandin E2 from arachidonic acid. Prostaglandins and related factors 32. *Biochim Biophys Acta 90*: 207–210
8. VAN DORP D A, BEERTHUIS R F, NUGTEREN D H, VON KEMAN H 1964 The biosynthesis of prostaglandins. *Biochim Biophys Acta 90*: 204–207
9. SMITH W L, LANDS W E 1972 Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. *Biochemistry 11(17)*: 3276–3285
10. MIYAMOTO T, OGINO N, YAMAMOTO S, HAYAISHI O 1976 Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem 251(9)*: 2629–2636

11. PAIRET M, ENGELHARDT G 1996 Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. *Fundam Clin Pharmacol 10(1):* 1–17
12. FLOWER 2006 Prostaglandins, bioassay and inflammation. *Br J Pharmacol 147(Suppl 1)*: S182–192
13. TSAI A-L, KULMACZ R J 2010 Prostaglandin H synthase: Resolved and unresolved mechanistic issues. *Arch Biochem Biophys 493(1)*: 103–124
14. FLOWER R J, VANE J R 1972 Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature 240(5381)*: 410–411
15. KUJUBU D A, FLETCHER B S, VARNUM B C, LIM R W, HERSCHMAN H R 1991 TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem 266(20):* 12866–12872
16. XIE W L, CHIPMAN J G, ROBERTSON D L, ERIKSON R L, SIMMONS D L 1991 Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA 88(7)*: 2692–2696
17. HLA T, NEILSON K 1992 Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A 89(16):* 7384–7388.
18. O'BANNION M K, WINN V D, YOUNG D A 1992 cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. *Proc Natl Acad Sci USA 89(11):* 4888–4892
19. BOTTING R M 2000 Mechanism of action of acetaminophen: is there a cyclooxygenase 3? Clin *Infect Dis 31 Suppl 5*: S202–210
20. CHANDRASEKHARAN N V, DAI H, ROOS K L, EVANSON N K, TOMSIK J, ELTON T S, SIMMONS D L 2002 COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA 99(21)*: 13926–13931
21. FUNK C D, FUNK L B, KENNEDY M E, PONG A S, FITZGERALD G A 1991 Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. *FASEB J 5*: 2304–2312
22. YOKOYAMA C, TANABE T 1989 Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. *Biochem Biophys Res Commun 165(2):* 888–894.
23. JONES D A, CARLTON DP , MCINTYRE T M, ZIMMERMANN G A, PRESCOTT SM 1993 Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. *J Biol Chem 268*: 9049–9054
24. APPLEBY S B, RISTIMAKI A, NEILSON K, NARKO K, HLA T 1994 Structure of the human cyclo-oxygenase-2 gene. *Biochem J 302*: 723–727
25. PICOT D, LOLL P J, GARAVITO R M 1994 The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature 367*: 243–249
26. KURUMBAIL R G, STEVENS A M, GIERSE J K, MCDONALD J J, STEGEMAN R A, PAK J Y, GILDEHAUS D, MIYASHIRO J M, PENNING T D, SEIBERT K, ISAKSON P C, STALLINGS W C 1996 Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature 384(6610)*: 644–648
27. LUONG C, MILLER A, BARNETT J, CHOW J, RAMESHA C, BROWNER M F 1996 Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol 3(11)*: 927–933
28. LU G, TSAI A L, VAN WART H E, KULMACZ R J 1999 Comparison of the peroxidase reaction kinetics of prostaglandin H synthase-1 and -2. *J Biol Chem 274(23)*: 16162–16167
29. LANDINO L M, CREWS B C, GIERSE J K, HAUSER S D, MARNETT L J 1997 Mutational analysis of the role of the distal histidine and glutamine residues of prostaglandin-endoperoxide synthase-2 in peroxidase catalysis, hydroperoxide reduction, and cyclooxygenase activation. *J Biol Chem 272(34)*: 21565–21574
30. KULMACZ R J, VAN DER DONK W A, TSAI A-L 2003 Comparison of the properties of prostaglandin H synthase-1 and -2. *Prog Lipid Res 42(5)*: 377–404
31. SMITH W L, LANGENBACH R 2001 Why there are two cyclooxygenase isozymes? *J Clin Invest 107(12)*: 1491–1495
32. O'NEILL G P, FORD-HUTCHINSON A W 1993 Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett 330*: 156–160
33. ZIMMERMANN K C, SARBIA M, SCHROR K, WEBER A A 1998 Constitutive cyclooxygenase-2 expression in healthy human and rabbit gastric mucosa. *Mol Pharmacol 54*: 536–540
34. BERNARDINI N, COLUCCI R, MATTII L, SEGNANI C, FORNAI M, DE GIORGIO R, BARBARA G, CASTAGNA M, NARDINI V, DOLFI A, DEL TACCA M, BLANDIZZI C 2006 Constitutive expression of cyclooxygenase-2 in the neuromuscular compartment of normal human colon. *Neurogastroenterol Motil 18(8):* 654–662
35. ZIDAR N, ODAR K, GLAVAC D, JERSE M, ZUPANC T, STAJER D 2008 Cyclooxygenase in normal human tissues—is COX-1 really a constitutive isoform, and COX-2 an inducible isoform? *J Cell Mol Med 13(9B)*: 3753–3763
36. WALLACE J L, MCKNIGHT W, REUTER B K, VERGNOLLE N 2000 NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology 119(3)*: 706–714
37. QI Z, CAI H, MORROW J D, BREYER M D 2006 Differentiation of cyclooxygenase 1- and 2-derived prostanoids in mouse kidney and aorta. *Hypertension 48(2)*: 323–328
38. QIN N, ZHANG S-P, REITZ T L, MEI J M, FLORES C M 2005 Cloning, expression, and functional characterization of human cyclooxygenase-1 splicing variants: evidence for intron 1 retention. *J Pharmacol Exp Ther 315(3)*: 1298–1305
39. VANE J R 1971 Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol 231(25):* 232–235
40. GROEGER A L, CIPOLLINA C, COLE M P, WOODCOCK S R, BONACCI G, RUDOLPH T K, RUDOLPH V, FREEMAN B A, SCHOPFER F J 2010 Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. *Nat Chem Biol 6(6):* 433–441
41. HARRIS R E 2009 Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. *Inflammopharmacology 17*: 55–67
42. WANG D, DUBOIS R N 2009 The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene 29(6)*: 781–788
43. SOBOLEWSKI C, CERELLA C, DICATO M, GHIBELLI L, DIEDERICH M 2010 The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol 2010:* 215158
44. MANTOVANI A, ALLAVENA P, SICA A, BALKWILL F 2008 Cancer-related inflammation. *Nature 454(7203*): 436–444
45. MBONYE U R, SONG I 2009 Posttranscriptional and posttranslational determinants of cyclooxygenase expression. *BMB Rep 42(9):* 552–560
46. BENNETT A, CIVIER A, HENSBY C N, MELHUISH P B, STAMFORD I F 1987 Measurement of arachidonate and its metabolites extracted from human normal and malignant gastrointestinal tissues. *Gut 28(3)* :315–318
47. WANG M T, HONN K V, NIE D 2007 Cyclooxygenases prostanoids and tumor progression. *Cancer Metastasis Rev 26*: 525–534
48. GREENHOUGH A, SMARTT H J M, MOORE A E, ROBERTS H R, WILLIAMS A C, PARASKEVA C, KAIDI A 2009 The COX-2/PGE_2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenviroment. *Carcinogenesis 30(3)*: 377–386
49. WANG D, DUBOIS R N 2010 Eicosanoids and cancer. *Nat Rev Cancer 10(3)*: 181–193
50. LIU C H, CHANG S H, NARKO K, TRIFAN O C, WU M T, SMITH E, HAUDENSCHILD C, LANE T F, HLA T 2001 Over-expression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem 276*: 18563–18569
51. MULLER-DECKER K, NEUFANG G, BERGER I, NEUMANN M, MARKS F, FURSTENBERGER G 2002 Transgenic cyclooxygenase-2 over-expression sensitizes mouse skin for carcinogenesis. *Proc Natl Acad Sci USA 99*: 12483–12488
52. AL-SALIHI M A, TERRECE PEARMAN A, DOAN T, REICHERT E C, ROSENBERG D W, PRESCOTT S M, STAFFORINI D M, TOPHAM M K 2009 Transgenic expression of cyclooxygenase- 2 in mouse intestine epithelium is insufficient to initiate tumorigenesis but promotes tumor progression. *Cancer Lett 273*: 225–232
53. OSHIMA M, DINCHUK J E, KARGMAN S L, OSHIMA H, HANCOCK B, KWONG E, TRZASKOS J M, EVANS J F, TAKETO M M 1996 Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell 87(5):* 803–809
54. SUBBARAMAIAH K, NORTON L, GERALD W, DANNENBERG A J 2002 Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. *J Biol Chem 277(21)*: 18649–18657
55. NEIL J R, JOHNSON K M, NEMENOFF R A, SCHIEMANN W P 2008 Cox-2 inactivates Smad signaling and enhances EMT

stimulated by TGF-beta through a PGE2-dependent mechanisms. *Carcinogenesis29(11):* 2227–2235

56. TSUJII M, KAWANO S, DUBOIS R N 1997 Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A 94(7):* 3336–3340
57. BUCHANAN F G, WANG D, BARGIACCHI F, DUBOIS R N 2003 Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *J Biol Chem 278(37*): 35451–35457
58. CASTELLONE M D, TERAMOTO H, WILLIAMS B O, DRUEY K M, GUTKIND J S 2005 Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science 310*: 1504–1510
59. SINICROPE F A, GILL S 2004 Role of cyclooxygenase-2 in colorectal cancer. *Cancer Metastasis Rev 23(1–2)*: 63–75
60. WANG D, WANG H, BROWN J, DAIKOKU T, NING W, SHI Q, RICHMOND A, STRIETER R, DEY S K, DUBOIS R N 2006 CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. *J Exp Med 203(4):* 941–951
61. ARENBERG D A, POLVERINI P J, KUNKEL S L, SHANAFELT A, HESSELGESSER J, HORUK R, STRIETER R M 1997 The role of CXC chemokines in the regulation of angiogenesis in non-small cell lung cancer. *J Leukoc Biol 62*: 554–562
62. LANE B R, LIU J, BOCK P J, SCHOLS D, COFFEY M J, STRIETER R M, POLVERINI P J, MARKOVITZ D M 2002 Interleukin-8 and growthregulated oncogene alpha mediate angiogenesis in Kaposi's sarcoma. *J Virol* 76: 11570–11583
63. GHOSH N, CHAKI R, MANDAL V, MANDAL S C 2010 COX-2 as a target for cancer chemotherapy. *Pharmacol Rep 62(2)*: 233–244
64. GALLICCHIO L, MCSORLEY M A, NEWSCHAFFER C J, THUITA L W, HUANG H Y, HOFFMAN S C, HELZLSOUER K J 2006 Nonsteroidal antiinflammatory drugs, cyclooxygenase polymorphisms, and the risk of developing breast carcinoma among women with benign breast disease. *Cancer 106*: 1443–1452
65. VOGEL U, CHRISTENSEN J, WALLIN H, FRIIS S, NEXØ B A, RAASCHOU-NIELSEN O, OVERVAD K, TJØNNELAND A 2008 Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. *Mutat Res 639*: 89–100
66. COX D G, BURING J, HANKINSON S E, HUNTER D J 2006 A polymorphism in the 3' untranslated region of the gene encoding prostaglandin endoperoxide synthase 2 is not associated with an increase in breast cancer risk: a nested case-control study. *Breast Cancer Res 9(1)*:R3
67. VOGEL U, CHRISTENSEN J, WALLIN H, FRIIS S, NEXO B A, TJONNELAND A Polymorphisms in COX-2, NSAID use and risk of basal cell carcinoma in a prospective study of Danes. *Mutat Res 617*: 138–146.
68. DONG J, DAI J, ZHANG M, HU Z, SHEN H 2010 Potentially functional COX-2-1195G>A polymorphism increases the risk of digestive system cancers: a meta-analysis. *J Gastroenterol Hepatol 25(6):* 1042–1050
69. SHENG H, WILLIAMS C S, SHAO J, LIANG P, DUBOIS R N, BEAUCHAMP R D 1998 Induction of cyclooxygenase-2 by activated Ha-ras oncogene in Rat-1 fibroblasts and the role of mitogen-activated protein kinase pathway. *J Biol Chem 273(34):* 22120–22127
70. SHENG H, SHAO J, DIXON D A, WILLIAMS C S, PRESCOTT S M, DUBOIS R N, BEAUCHAMP R D 2000 Transforming growth factor-beta1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. *J Biol Chem 275(9)*: 6628–6635
71. SUBBARAMAIAH K, ALTORKI N, CHUNG W J, MESTRE J R, SAMPAT A, DANNENBERG A J 1999 Inhibition of cyclooxygenase-2 gene expression by p53. *J Bio. Chem 274*: 10911–10915
72. WU W K, SUNG J J, LEE C W, YU J, CHO C H 2010 Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. *Cancer Lett 295(1)*:7–16
73. TOYOTA M, SHEN L, OHE-TOYOTA M, HAMILTON S R, SINICROPE F A, ISSA J P 2000 Aberrant Methylation of the Cyclooxygenase 2 CpG Island in Colorectal Tumors. *Cancer Res 60: 4044–4048*
74. KIRTIKARA K, MORHAM S G, RAGHOW R, LAULEDERKIND S J, KANEKURA T, GOORHA S, BALLOU L R 1998 Compensatory prostaglandin E2 biosynthesis in cyclooxygenase 1 or 2 null cells. *Exp Med 187(4)* :517–523
75. ZILLI M, IACOBELLI S 2010 Chemoprophylaxis in gastrointestinal tumors. *Eur Rev Med Pharmacol Sci 14(4)*: 285–291
76. LEE C R, BOTTONE F G J R, KRAHN J M, LI L, MOHRENWEISER H W, COOK M E, PETROVICH R M, BELL D A, ELING T E, ZELDIN D C 2007 Identification and functional characterization of polymorphisms in human cyclooxygenase-1 (PTGS1). *Pharmacogenet Genomics 17(2):*145–160
77. ARISAWA T, TAHARA T, SHIBATA T, NAGASAKA M, NAKAMURA M, KAMIYA Y, FUJITA H, NAKAMURA M, YOSHIOKA D, ARIMA Y, OKUBO M, HIRATA I, NAKANO H 2007 Association between genetic polymorphisms in the cyclooxygenase-1 gene promoter and peptic ulcers in Japan. *Int J Mol Med 20(3)*: 373–378
78. ULRICH C M, BIGLER J, SPARKS R, WHITTON J, SIBERT J G, GOODE E L, YASUI Y, POTTER J D 2004 Polymorphisms in PTGS1 (=COX-1) and Risk of Colorectal Polyps. *Cancer Epidemiol Biomarkers Prev 13(5):* 889–893
79. FUJITA T, MATSUI M, TAKAKU K, UETAKE H, ICHIKAWA W, TAKETO M M, SUGIHARA K 1998 Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. *Cancer Res 58(21):* 4823–4826
80. KARGMAN S L, O'NEILL G P, VICKERS P J, EVANS J F, MANCINI J A, JOIHY S1995 Expression of prostaglandin (i/H synlhase-1 and -2 protein in human colon cancer. *Cancer Res 55*: 2556–2559
81. FORNAI M, BLANDIZZI C, COLUCCI R, ANTONIOLI L, BERNARDINI N, SEGNANI C, BARAGATTI B, BAROGI S, BERTI P, SPISNI R, DEL TACCA M 2005 Role of cyclooxygenases 1 and 2 in the modulation of neuromuscular functions in the distal colon of humans and mice. *Gut 54*: 608–616
82. EBERHART C E, COFFEY R J, RADHIKA A, GIARDIELLO F M, FERRENBACH S, DUBOIS R N 1994 Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology 107(4):* 1183–1188
83. CHULADA P C, THOMPSON M B, MAHLER J F, DOYLE C M, GAUL B W, LEE C, TIANO H F, MORHAM S G, SMITHIES O, LANGENBACH R 2000 Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res 60*: 4705–4708
84. SANO H, KAWAHITO Y, WILDER R L, HASHIRAMOTO A, MUKAI S, ASAI K, KIMURA S, KATO H, KONDO M, HLA T 1995 Expression of Cyclooxygenase-1and -2 in Human Colorectal Cancer. *Cancer Res 55(17):* 3785–3789
85. SHEEHAN K M, SHEAHAN K, O'DONOGHUE D P, MACSWEENEY F, CONROY R M, FITZGERALD D J, MURRAY F E 1999 The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA 282(13):* 1254–1257
86. MASUNAGA R, KOHNO H, DHAR D K, OHNO S, SHIBAKITA M, KINUGASA S, YOSHIMURA H, TACHIBANA M, KUBOTA H, NAGASUE N 2000 Cyclooxygenase-2 expression correlates with tumor neovascularization and prognosis in human colorectal carcinoma patients. *Clin Cancer Res 6(10):* 4064–4068
87. TOMOZAWA S, TSUNO N H, SUNAMI E, HATANO K, KITAYAMA J, OSADA T, SAITO S, TSURUO T, SHIBATA Y, NAGAWA H 2000 Cyclooxygenase-2 overexpression correlates with tumour recurrence, especially haematogenous metastasis, of colorectal cancer. *Br J Cancer 83(3):* 324–328
88. PRESCOTT S M, FITZPATRICK F A Cyclooxygenase-2 and carcinogenesis. 2000 Biochim *Biophys Acta 1470(2):* M69-78
89. KUTCHERA W, JONES D A, MATSUNAMI N, GRODEN J, MCINTYRE T M, ZIMMERMAN G A, WHITE R L, PRESCOTT S M 1996 Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: Evidence for a transcriptional effect. *Proc Natl Acad Sci U S A 93(10)*: 4816–4820
90. MAEKAWA M, SUGANO K, SANO H, MIYAZAKI S, USHIAMA M, FUJITA S, GOTODA T, YOKOTA T, OHKURA H, KAKIZOE T, SEKIYA T 1998 Increased expression of cyclooxygenase-2 to -1 in human colorectal cancers and adenomas, but not in hyperplastic polyps. *Jpn J Clin Oncol 28(7):* 421–426
91. HAO X, BISHOP A E, WALLACE M, WANG H, WILLCOCKS T C, MACLOUF J, POLAK J M, KNIGHT S, TALBOT I C 1999 Early expression of cyclo-oxygenase-2 during sporadic colorectal carcinogenesis. *J Pathol 187(3):* 295–301
92. DIMBERG, SAMUELSSON, HUGANDER, SODERKVIST P 1999 Differential expression of cyclooxygenase 2 in human colorectal cancer. *Gut 45*: 730–732
93. SINICROPE F A, LEMOINE M, XI L, LYNCH P M, CLEARY K R, SHEN Y, FRAZIER M L 1999 Reduced expression of cyclooxy-

genase 2 proteins in hereditary nonpolyposis colorectal cancers relative to sporadic cancers. *Gastroenterology 117*: 350–358

94. GOODMAN J E, BOWMAN E D, CHANOCK S J, ALBERG A J, HARRIS C C 2004 Arachidonate lipoxygenase (ALOX) and cyclooxygenase (COX) polymorphisms and colon cancer risk. *Carcinogenesis 25(12):* 2467–2472

95. THOMPSON C L, PLUMMER S J, MERKULOVA A, CHENG I, TUCKER T C, CASEY G, LI L 2009 No association between cyclooxygenase-2 and uridine diphosphate glucuronosyltransferase 1A6 genetic 2. *World J Gastroenterol 15(18)*: 2240–2244

96. LIN H J, LAKKIDES K M, KEKU T O, REDDY S T, LOUIE A D, KAU I H, ZHOU H, GIM J S, MA H L, MATTHIES C F, DAI A, HUANG H F, MATERI A M, LIN J H, FRANKL H D, LEE E R, HARDY S I, HERSCHMAN H R, HENDERSON B E, KOLONEL L N, LE MARCHAND L, GARAVITO R M, SANDLER R S, HAILE R W, SMITH W L 2002 Prostaglandin H synthase 2 variant (Val511Ala) in African Americans may reduce the risk for colorectal neoplasia. *Cancer Epidemiol Biomarkers Prev 11(11):* 1305–1315

97. BARRY E L, SANSBURY L B, GRAU M V, ALI I U, TSANG S, MUNROE D J, AHNEN D J, SANDLER R S, SAIBIL F, GUI J, BRESALIER R S, MCKEOWN-EYSSEN G E, BURKE C, BARON J A 2009 Cyclooxygenase-2 polymorphisms, aspirin treatment, and risk for colorectal adenoma recurrence—data from a randomized clinical trial. *Cancer Epidemiol Biomarkers Prev 18(10)*: 2726–2733

98. COX D G, PONTES C, GUINO E, NAVARRO M, OSORIO A, CANZIAN F, MORENO V; BELLVITGE COLORECTAL CANCER STUDY GROUP 2004 Polymorphisms in prostaglandin synthase 2/cyclooxygenase 2 (PTGS2/COX2) and risk of colorectal cancer. *Br J Cancer 91(2):* 339–343

99. IGLESIAS D, NEJDA N, AZCOITA M M, SCHWARTZ S J R, GONZÁLEZ-AGUILERA J J, FERNÁNDEZ-PERALTA A M 2009 Effect of COX2 -765G>C and c.3618A>G polymorphisms on the risk and survival of sporadic colorectal cancer. *Cancer Causes Control 20(8):* 1421–1429

100. PEREIRA C, MEDEIROS R M, DINIS-RIBEIRO M J 2009 Cyclooxygenase polymorphisms in gastric and colorectal carcinogenesis: are conclusive results available? *Eur J Gastroenterol Hepatol 21(1)*: 76–91

101. TAKETO M M 1998 Cyclooxygenase-2 Inhibitors in Tumorigenesis. *J Natl Cancer Inst 90(21)*: 1609–1620

102. STRILLACCI A, GRIFFONI C, VALERII M C, LAZZARINI G, TOMASI V, SPISNI E 2010 RNAi-based strategies for cyclooxygenase-2 inhibition in cancer. *J Biomed Biotechnol 2010*: 828045

103. KOEBERLE A, WERZ O 2009 Inhibitors of the microsomal prostaglandin E(2) synthase-1 as alternative to non steroidal anti-inflammatory drugs (NSAIDs)—a critical review. *Curr Med Chem 16(32)*: 4274–4296