INTRODUCTION

Cyclooxygenase (COX) or prostaglandin endoperoxide synthase (PGHS) is the key and rate-limiting enzyme that catalyses the initial step of arachidonic acid (AA) metabolic transformation into prostanoids (prostacycline, thromboxane, prostaglandins E2, D2, F2α). Sir John Vane was the first to demonstrate in 1971 that non-steroid anti-inflammatory drugs (NSAIDs) block cyclooxygenase, then five years later, COX was isolated, and cloned in 1988 by three separate groups.1-4

Initially, cyclooxygenases were believed to be expressed constitutively with constant levels in individual tissues; however, cyclooxygenase activity increases in inflammation, increase prevented by corticosteroids. Consequently, between 1989 and 1992 was identified a new isoform cyclooxygenase-2 (COX-2), by two different approaches.5 This gave rise to the concept of "constitutive" cyclooxygenase-1 (COX-1) and "inducible" COX-2 forms of COX. Both isoforms COX-1 and COX-2 are now well known and characterized cyclooxygenases. The elucidation of the structure and the cloning of the gene for COX-2 were of great support to illuminate the understanding of non steroidal anti-inflammatory drugs (NSAIDs) pharmacology.6

A splice variant of COX-1 was isolated in 2002 and has been called cyclooxygenase-3, (COX-3), COX-1b or COX-1v. The expression of COX-3 mRNA was detected in human cerebral cortex and this protein is pharmacologically different to COX-1 and COX-2 even it is derived from the COX-1 gene.7,8

The discovery of COX-2 isoform and the current understanding of its contribution to tumorigenesis opened a new frontier in cancer research. The
objective of this review is to discuss in the light of the newest data literature the molecular and biochemical features of COX isoforms, the contributions of COX-2 to tumorigenesis, as well as, the possible role of COX-2 inhibition as a target for anticancer drug development.

**CYCLOOXYGENASE-1**

The human gene for the COX-1 is of 22 kilobase and is located on the chromosome 9 and consists of 11 exons and 10 introns.9

Cyclooxygenase-1, the prostaglandin endoperoxide synthase-1, is the constitutive isoform of COX, and shares about 60% homology at the aminoacid level with COX-2.10,11 COX-1 is a membrane-bound protein residing in the endoplasmic reticulum of prostanoid forming cells, and has a molecular mass of 71 kDa.12

The aminoacids in its structure form a long narrow channel that ends with a hairpin-like bend at the end.13,14 The aminoacids at the positions 120, 530 and 385 are arginine, serine and tyrosine, found also in the structure of COX-2, are the binding sites for the NSAIDs and the arachidonic acid.13 The arginine 120 is positioned close to the opening of the COX channel and the 530 serine and the 385 tyrosine are positioned at the apex of the long active site. The aminoacids at position 434 and 523 are responsible for the selective inhibition of these enzymes by NSAIDs; in COX-1 is an 434, 523 isoleucine which blocks by steric hindrance, the access of the COX-2-selective NSAIDs while in COX-2 is a valine which is smaller than isoleucine and gives access to the pocket-like binding site of the COX-2 selective NSAIDs.13

The substrate for COX-1 is the arachidonic acid, and COX-1 has a lower ability than COX-2 to compete for this substrate, when AA is present in low concentration.9

COX-1 forms the endoperoxide derivatives, prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2) using identical enzymatic processes as COX-2 and the same COX-2 receptors on the same target tissues and cells, are used to mediate the effects of the released prostanoids.9

**CYCLOOXYGENASE-2**

Although COX-1 and COX-2 are remarkably similar in many respects, important differences distinguish the two isozymes.

The COX-2 gene for human is approximately 8.3 kilobase long, consist of 10 exons and 9 introns and is located on chromosome 1.11 The difference between COX-1 and COX-2 gene is the presence of an additional small intron (intron 1) located at the 5' end of the COX-1 gene. This "extra" intron of 94 nucleotides in the human COX-1 gene as well as the fact that the intron/exon boundaries of the COX-1 gene are the same as in the COX-2 gene, strongly support the hypothesis that they have descended from an ancestral gene, via gene duplication.15,16

In 1989 Needleman et al. reported that bacterial lipopolysaccharide increased the synthesis of prostaglandins in human monocytes in vitro, and only this increase was inhibited by dexamethasone, but not also the basal level of the enzyme.17 This was associated with de novo synthesis of a new COX protein and consequently appeared the concept of an "inducible" form of COX.

Cyclooxygenase-2, or prostaglandin endoperoxide synthase-2, is the inducible form of the COX. Both isoforms COX-1 and COX-2 have a molecular weight of 71 kDa and the aminoacid sequence of COX-2 shows about 60% homology with the non-inducible enzyme.10,15,19 The mRNA for the inducible enzyme approximates 4.5 kb and that of the constitutive enzyme 2.8 kb.17 COX-1 and COX-2 are membrane-bound proteins that reside, after synthesis and transport, initially in the endoplasmic reticulum (ER). COX-2 is mainly located in the lumen of the ER, where the oxidizing conditions favors its proper dimerization, and in the nuclear envelopes.18

The COX-2 gene encodes for a homodimeric protein of 604 aminoacids in size, which is highly similar in structure and enzymatic activity to COX-1.20 In contrast to COX-1, the COX-2 promoter is not basally active in most cell types, but can be strongly and rapidly induced by growth factors and pro-inflammatory mediators. COX-2 is undetectable in most normal tissues (except for the central nervous system, kidneys, and seminal vesicles) but is induced by various inflammatory and mitogenic stimuli. Growth factors (epidermal growth factor, platelet derived growth factor, pro-inflammatory cytokines (IL-1β, IL-2) and tumor necrosis factor, tumor promoters, bile acids and ultraviolet B irradiation are all stimulators of COX-2 expression.21-24

X-ray crystallography analysis revealed that the tertiary structures of COX-1 and COX-2 are very similar and the aminoacids form a long-narrow channel with a hairpin bend at the end.5 Even if the aminoacid conformation for the substratebinding site and the catalytic regions are almost identical (arginine 120, serine 530 and tyrosine 385 positioned in this order in COX-2, correspond to the opening to the apex of the narrow-channel), there are
The receptors which determine the effects for the prostanoids released as described above, are cell-membrane spanning G-protein-coupled receptors and five major subdivisions were pharmacologically defined, each corresponding to one of the COX metabolites: DP for PGD2, EP for PGE2, FP for PGF2α, IP for PGI2 and, TP for TXA2.10

A summary of the prostanoid synthesis involving COX-1, COX-2 and COX-3 enzymes and the two main effects caused by their NSAIDs inhibition is presented in Figure 1.

**CYCLOOXYGENASE-3**

Cyclooxygenase-3 is termed a splice variant of COX-1. It was isolated by Chandrasekharan et al. in 2002 from the heart and the cerebral cortices of the dog.7 The expression of COX-3 mRNA in humans was found to be expressed as an about 5.2 kb transcript and it was found to be most abundant in cerebral cortex and heart.10

The human COX-3, a protein made from 633 aminoacids, is a membrane-bound enzyme and shares all the catalytic and structural properties of COX-1 and COX-2.7 It is made from a COX-1 gene which retains intron 1 and is made up of 94 nucleotides in its mRNA.7,10,28

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**Figure 1.** The side effects of NSAIDs are caused by the inhibition of the constitutive enzyme COX-1. The therapeutic effects of NSAIDs are due to the inhibition of the inducible enzyme COX-2.

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**Membrane Phospholipids**

- **Arachidonic Acid**
  - **COX-3**
    - **COX-1** (Constitutive)
      - NSAIDs inhibit
        - **TXA2** (Platelets)
        - **PGI2** (Gastric Mucosa)
        - **PGE2** (Kidney)
    - **COX-2** (Induced)
      - COXIBs inhibit
        - Prostaglandins
          - Inflammation
            - Pain, Swelling

**NSAID: SIDE EFFECTS**

**THERAPEUTIC EFFECTS**
The inducible COX-3 is less potent and produces less PGE2 than either COX-1 and COX-2 and NSAIDs exhibit different inhibition potencies for COX-3 depending on their polarity and capacity to penetrate the blood brain barrier.\textsuperscript{29,30}

Antipyretic analgesic drugs (e.g. paracetamol), which are weak inhibitors of COX-1 and COX-2, and penetrate easily into the central nervous system, could explain their pharmacological action of inhibiting COX-3.\textsuperscript{29,30}

Research revealed that COX-3 may have a role in remission periods in chronic inflammatory disease and may be involved in the development of ovarian, cervical, colonic cancer and leukaemia.\textsuperscript{29}

**COX-2 AND CANCER**

Clinical and epidemiological data, and moreover, findings extrapolated from experimental studies, in cultured tumor cells and animal tumor models, suggest the critical role of COX-2 in tumorigenesis.\textsuperscript{1,12} The mechanisms by which COX-2 contributes to tumorigenesis are complex and not yet well understood. COX-2 is related to the formation of carcinogens, tumor promotion and inhibition of apoptosis, angiogenesis and the metastatic process.\textsuperscript{33} In addition, large epidemiological studies have shown indications that COX inhibitors could have a beneficial influence on diminishing the development and growth of malignancies.\textsuperscript{34}

The activation of carcinogens due to COX-2 activity is a first step before tumor initiation. The metabolism of arachidonic acid produces mutagens (e.g. a by-product of the oxidation of AA, malondialdehyde, is highly chemically reactive and form adducts with DNA).\textsuperscript{35} The peroxydase activity of COX-2 can convert procarcinogens to carcinogens and initiate tumor formation. Numerous xenobiotics can be transformed by oxidation path into mutagens by peroxydase activity of COX. Because in the liver this oxidative reactions are mainly catalyzed by cytochrome P-450s, the mutagen formation is thus prevented. By contrast, the colon has low concentration of P-450s and others monoxygenases, leading to co-oxidation of significant number of xenobiotics to mutagens, by the peroxydase activity of COX-2.\textsuperscript{36,37}

Tumor initiation induced by COX-2 over-expression was first demonstrated by the studies of Liu et al.\textsuperscript{38} Their experimental findings in transgenic mice model implied for the first time that the sole expression of COX-2 is sufficient to initiate tumor formation. Research carried out by Crawford et al. suggests that COX-2 plays a tumor-initiating role in human breast cancer. They demonstrated in normal human epithelial mammary cells that expressed COX-2, the high disposition of these cells to transform into malignant cells.\textsuperscript{39}

Additional evidence in the study of Colby et al. in a mouse pancreas model shows that COX-2 expression was not sufficient to induce metastasis, unless tumor cells were subjected to additional insults, thus underscoring the link between chronic inflammation and tumor initiating effects of COX-2.\textsuperscript{40}

The mechanisms of tumor-initiating effects of COX-2 are not well elucidated. In the absence of clear-expressed signs of inflammation, COX-2 can function in a cell-autonomous manner transforming normal epithelial cells and thus conferring resistance to apoptosis. COX-2 itself via the production of reactive oxygen species and lipid endoperoxide products can induce genomic instability and mutagenic effects.\textsuperscript{41} The presence of a chronic inflammatory process initiated and maintained by COX-2, can constitute the determinant for the tumor-initiating effect of the enzyme. COX-2-derived prostaglandins contribute to tumor growths by inducing angiogenesis that sustains tumor cell viability and growth. Stimulation of angiogenesis by COX-2 has also an important role in stimulation and progression of inflammation.

The role of COX-2 in tumor promotion is strongly supported by many clinical and experimental studies in colorectal tumor models.\textsuperscript{42-44} Most of the results support the concept that COX-2 exerts tumor-promoting effects in a context-dependent manner. However, recent work of Ishikawa et al. demonstrated that neither COX-2 nor COX-1 are required for the development of colon cancer in a mouse model of colitis-associated colon cancer.\textsuperscript{45}

Various experimental studies have shown a positive correlation between the expression of COX-2 and inhibition of apoptosis. The inhibition of apoptosis is determined by the over-expression of COX-2 in tumor cells which increase the proto-oncogene Bel-2 family which consists of 20 homologues of important pro- and anti-apoptotic regulators of programmed cell deaths.\textsuperscript{46,47}

The contribution of COX-2 in invasion and metastasis, which are the most insidious and life-threatening aspects of cancer, was first reported by Tsuji et al. In their studies, using cultured colon human cell lines, they demonstrated that forced expression of COX-2 increases metastatic potential also revealed by subsequent experimental studies in models of breast and lung cancers. COX-2 was shown to be among the genes up-regulated in invasive breast cancer cells.\textsuperscript{48,50}
The mechanisms that determine the spread of cancer cells require sequential events: increased motility and invasive properties, invasion of the peripheral and lymphatic circulation, homing and colonization of distant organs. Each of the above described steps are influenced by COX-2, demonstrated by many evidences. In addition to influencing motility and invasion via several mechanisms, COX-2 was shown to be associated with elevated expression of metalloprotease-2 and, moreover prevents anoikis, and thus positively, sustains survival of tumor cells during metastasis.

It has been extensively documented that overexpression of COX-2 is implicated in various forms of human cancer, such as cancer of the lung, breast, colorectal, in particular, with tumor metastasis in colon cancer, prostate.

Coxibs are selective-inhibitors of COX-2 that irreversibly bind to the catalytic active site of the COX-2, leading to its inactivation, and these compounds, demonstrated marked antitumoral properties in cultured tumor cell-lines and tumor xenografts.

Within the family of coxibs, developed as anti-inflammatory agents, celecoxib has been most often investigated for its anti-proliferative effects in vitro, as well as for in vivo. However, recent data performed by a complex study, conclude that the anti-proliferative effect of celecoxib is not a class effect of coxibs, and that celecoxib represents a potentially unique drug within the coxibs group (rofecoxib, etoricoxib, valdecoxib, lumiracoxib, celecoxib), due to its anti-proliferative activity.

CONCLUSIONS

As described in this review, the constitutive COX-1 and inducible COX-2 dichotomy is giving way a more complex picture including the COX-1 spliced variant, each of one acting within a specialized pathway. In this review we have tried to encompass the role of COX-2 in cancer and its value as a target in cancer therapy. Several aspects of the mechanisms of COX-2 contribution to tumorigenesis were discussed and the anti-proliferative potency of COX-2 selective inhibitor, celecoxib was revealed. However, additional basic research is absolutely needed for a better understanding of the role of COX-2 in oncogenesis to determine when COX-2 is a cause or a consequence of neoplastic transformation. COX-2 over-expression is clear associated with carcinogenesis in many cancers and the inhibition of the enzyme may be a promising strategy which can provide beneficial effects for cancer patients.

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