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Review

Cyclooxygenases: Proliferation and differentiation

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Prostaglandins are formed from arachidonic acid by the action of cyclooxygenase and subsequent downstream synthetases. Mainly two cyclooxygenase isoforms have been identified which are now known as cyclooxygenase-1 and cyclooxygenase-2. Both iso-enzymes transform arachidonic acid to prostaglandins, but differ in their distribution and their physiological roles. The two isoenzymes are similar in protein structure but are produced by divergent genes and have different biological functions. Cyclooxygenase-1 is a constitutively expressed enzyme in most mammalian tissues and maintains normal cellular physiological functions, such as platelet aggregation and gastric cytoprotection; while cyclooxygenase-2 is normally expressed at a very low level in most tissues and is highly inducible by growth factors, cytokines, and tumour promoters. In several studies, the effect of cyclooxygenases on different cell types has been investigated. This review focuses on cyclooxygenases function, cell proliferation and differentiation.

Key words: Cyclooxygenases, proliferation, differentiation, prostaglandins, tumor.

INTRODUCTION

The prostaglandin H2 synthetases, which are membrane-bound enzymes, are members of the mammalian heme-dependent peroxidase family. Cyclooxygenases are the rate-limiting enzymes that catalyze the alteration of arachidonic acid released from membrane phospholipids to prostaglandin H2 and reactive oxygen species. Mainly, two cyclooxygenase isoforms, cyclooxygenase-1 and cyclooxygenase-2 have been recognized (Figures 1 and 2).

Both are homodimeric, glycosylated, and heme containing proteins with 2 catalytic sites (Mehmet et al., 2008). The two isoenzymes are similar in protein structure but are produced by divergent genes and have different biological functions. Cyclooxygenase-1 (cox-1) is a constitutively expressed enzyme in most mammalian tissues and maintains normal cellular physiological functions, such as platelet aggregation and gastric cytoprotection (Mehmet et al., 2008) while cyclooxygenase-2 is normally expressed at a very low level in most tissues and is highly inducible by growth factors, cytokines, and tumour promoters. Cyclooxygenase-2 (cox-2) and perhaps cox-1 have powerful effects on cell growth and survival (Figure 3).

Cyclooxygenase-2 is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation. Cyclooxygenase iso-enzymes have direct roles in many human pathological processes. These include thrombosis, inflammation, pain and fever, various cancers, and neurological disorders, such as Alzheimer and Parkinson diseases. Cyclooxygenase-2 inhibitors have been used to block angiogenesis and rapid expansion of tumours.

More recently, another cyclooxygenase enzyme, cyclooxygenase-3, was reported to be a splice variant of cyclooxygenase-1. Cyclooxygenase-3 is made from the cyclooxygenase-1 gene but retains intron 1 in its messenger RNA; in fact, cyclooxygenase-3 is a splice variant

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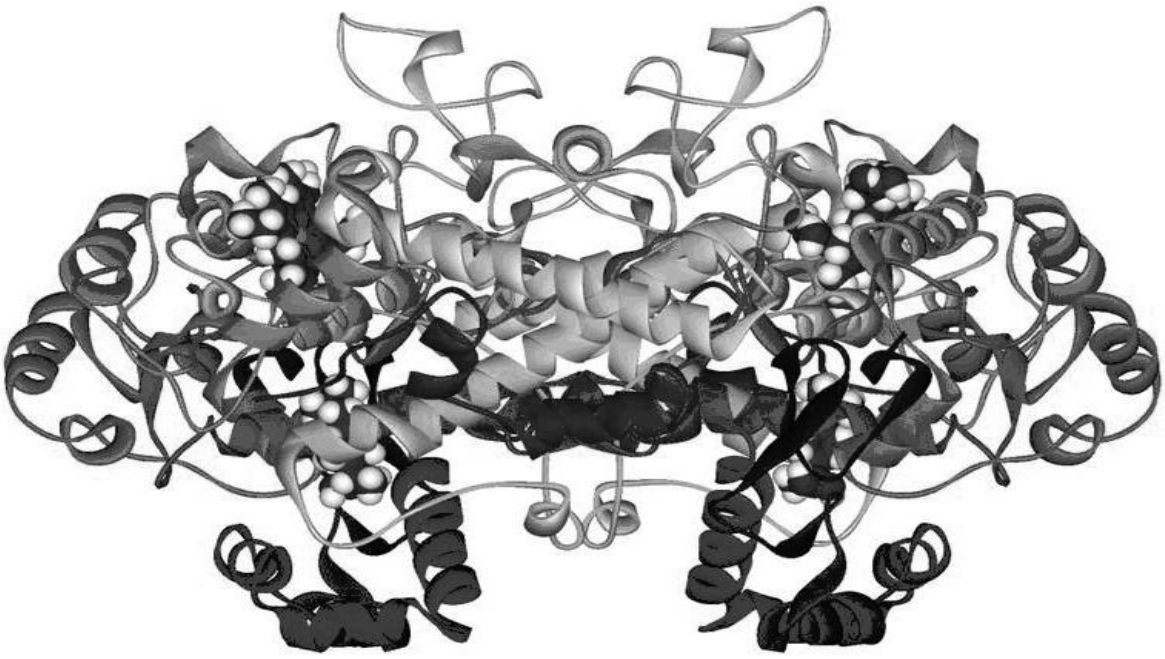


Figure 1. Diagram of cyclooxygenase-1.

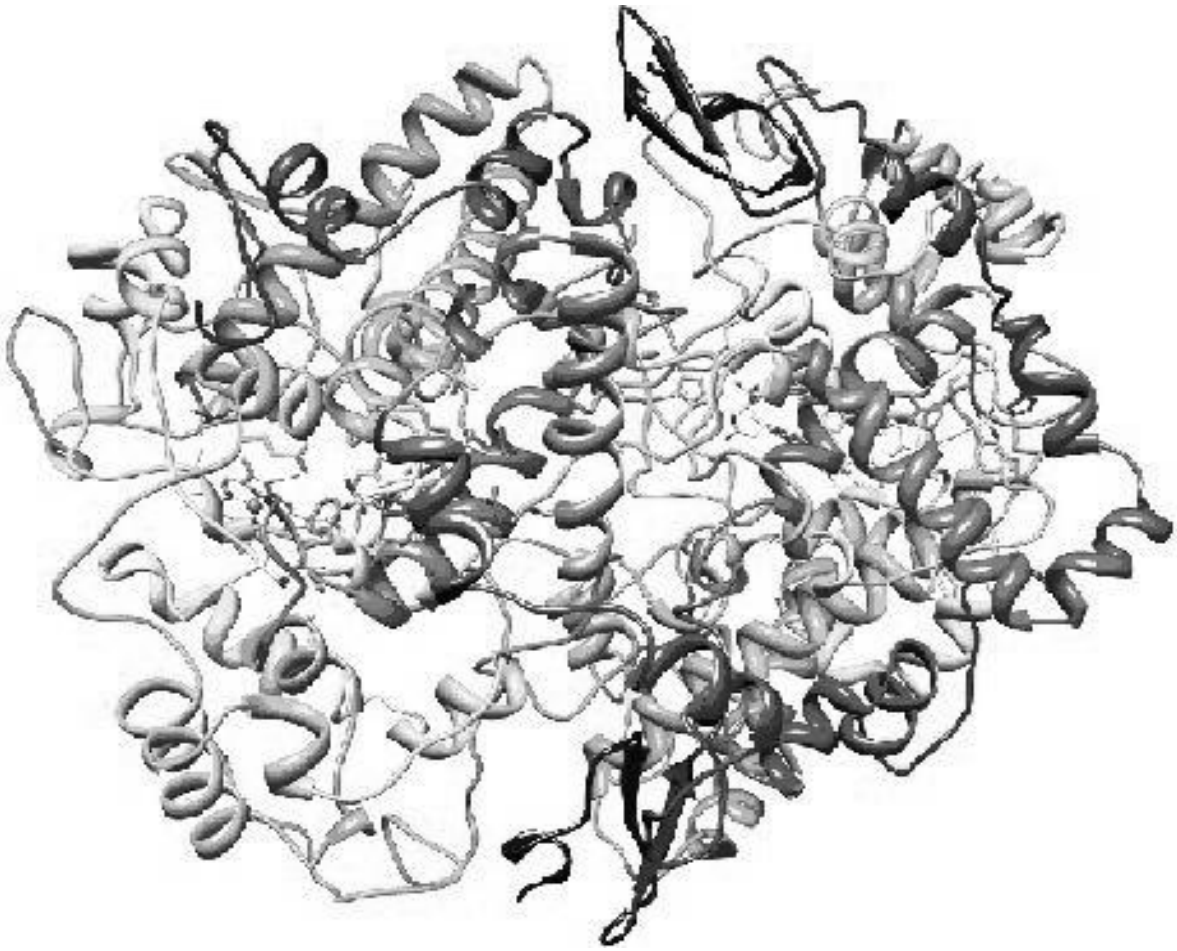


Figure 2. Diagram of cyclooxygenase-2.

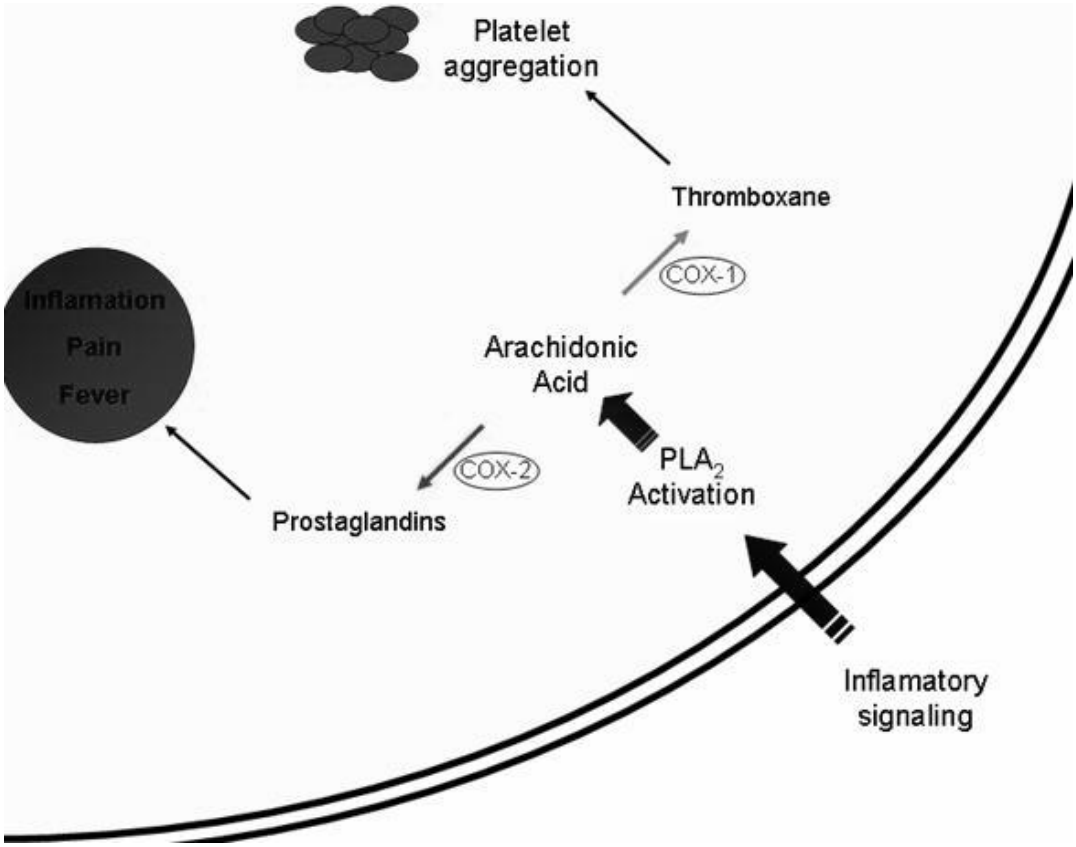


Figure 3. Mechanisms of the two different isoforms (cyclooxygenase-1 and cyclooxygenase-2).

of cyclooxygenase-1. In humans, cyclooxygenase-3 mRNA was found to be expressed as a 5.2 kb transcript that was most abundant in cerebral cortex and heart; the mRNA is presumably the cyclooxygenase-3 mRNA (Mehmet et al., 2008).

HISTORY OF CYCLOOXYGENASE

In 1976, Miyamoto and colleagues purified the cyclooxygenas -1 enzyme. Cyclooxygenase-2 was discovered by UCLA scientist Dr. Harvey Herschman in 1991 at the Daniel Simmons Laboratory, Brigham Young University. The enzyme was discovered in 1988 by Daniel Simmons, a Brigham Young University researcher, formerly of Harvard University. Dr. Daniel Simmons immediately understood the importance of his discovery. The same day the enzyme was sequenced, he had his notebook notarized as proof of his discovery. Subsequently, Pfizer, the research firm with whom Simmons had contracted, allegedly broke contract and refused to give Simmons any royalties and profits from his discovery. As of September 2010, a lawsuit was in progress by Simmons against the drug developers. An induced form of cyclooxygenase was described which was immunologically distinct from a constitutive enzyme. It was mitogenesis research, however, that led to the discovery of the cyclooxygenase-2 gene (Kujubu et al., 1991; Wong et al., 1991; Xie et al., 1991).

PROTEIN STRUCTURE OF THE CYCLOOXYGENASE ENZYMES

Both enzymes contain three high mannose oligosaccharides, one of which facilitates protein folding. A fourth oligosaccharide, present only in cyclooxygenase-2, regulates its degradation (Mehmet et al., 2008; Rouzer and Lawrence, 2007).

Each subunit of the dimmer consists of three domains, the epidermal growth factor domain, the membrane binding domain, and the catalytic domain comprising the bulk of the protein that contains the cyclooxygenase and peroxidase active sites on either side of the heme prosthetic group (Rouzer et al., 2007).

The proteins actually share 60% homology at the amino acid level. The primary structures in the beginning stages of the existence of cyclooxygenase-1 and cyclooxygenase-2 are 600 to 602 (depending on the species) and 604 amino acids, respectively, and both isoforms are then processed into mature forms by removal of signal peptides (Mehmet et al., 2008).

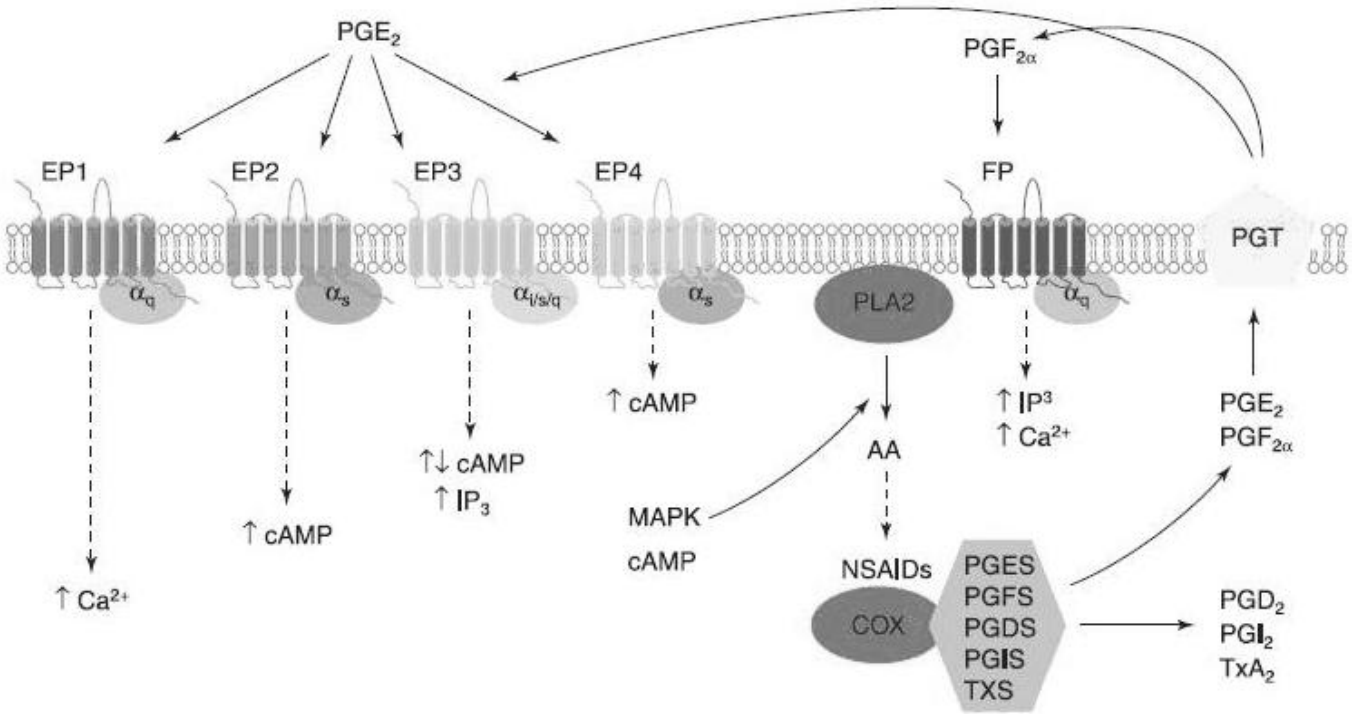


Figure 4. Schematic representation of the COX–prostanoid biosynthetic and signalling pathway (Kurt et al., 2003).

Molecular weight which mainly consists of glycosylation was observed as 68 kDa unmodified and about 75 to 80 kDa after post-translational modifications (Zhab et al., 2004). In spite of the similarities, the isoenzymes exhibit differences in patterns of tissue expression and cellular function (Mehmet et al., 2008).

CYCLOOXYGENASES AND PROSTANOIDS SYNTHETASE

Prostanoids, cyclooxygenase metabolites of arachidonic acid, consist of prostaglandin D2, prostaglandin E2, prostaglandin F2α, prostaglandin I2, and thromboxane A2 (Figure 4)(Mehmet et al., 2008).

Prostaglandin synthesis starts with the oxidative cyclization of the five carbons at the center of arachidonic acid, which is released by phospholipase A2 from the cell membrane. The free arachidonic acid is then presented to the endoplasmic reticulum and nuclear membrane, where the cyclooxygenase enzymes catalyze the rate-limiting step for prostaglandin synthesis, the generation of the biocyclic endoperoxide intermediate prostaglandin G2, and the reduction to prostaglandin H2. In different cell types and under different physiological conditions, the downstream metabolism of prostaglandin H2 can be dramatically different. Prostaglandin D synthase is usually found in mast cells and in the brain; prostaglandin F synthase is expressed in the uterus; prostaglandin I synthase is found in endothelial cells; thromboxane synthase is commonly seen in platelets and macrophages; and prostaglandin E isomerase appears in most cell types (Zhab et al., 2004).

Prostanoids activate cell and tissue specific receptors that belong to a subfamily of the G-protein-coupled receptor superfamily of 7 transmembrane spanning proteins. There are at least 9 known prostaglandin receptors. Prostaglandin E2 exerts its effects through interactions with EP1-EP4. In the same way, prostaglandin D2 binds to own receptors, DP1-DP2 subtypes. In contrast, prostaglandin F2α, prostaglandin I2, and thromboxane A2 have only single receptors FP, IP, and TP, respectively (Mehmet et al., 2008) and the nuclear peroxisome proliferator-activated receptors receptor class (that is, PPARα, PPARγ, PPARδ), that acts directly as a transcription factor upon ligand binding (Figure 5) (Dubois et al., 1998).

Functions of prostaglandins

Prostaglandins were first discovered in semen or in the extract of prostate as lipid soluble compounds with potent vasodepressor and smooth muscle-stimulating activity. They were named based on the fact that they were believed to be derived from the prostate (Zhab et al., 2004).

Prostaglandins are found in animals as primitive as the coelenterates and are present in a wide variety of human tissues. Prostaglandins not only play a central role in inflammation, but also regulate other critical physiological

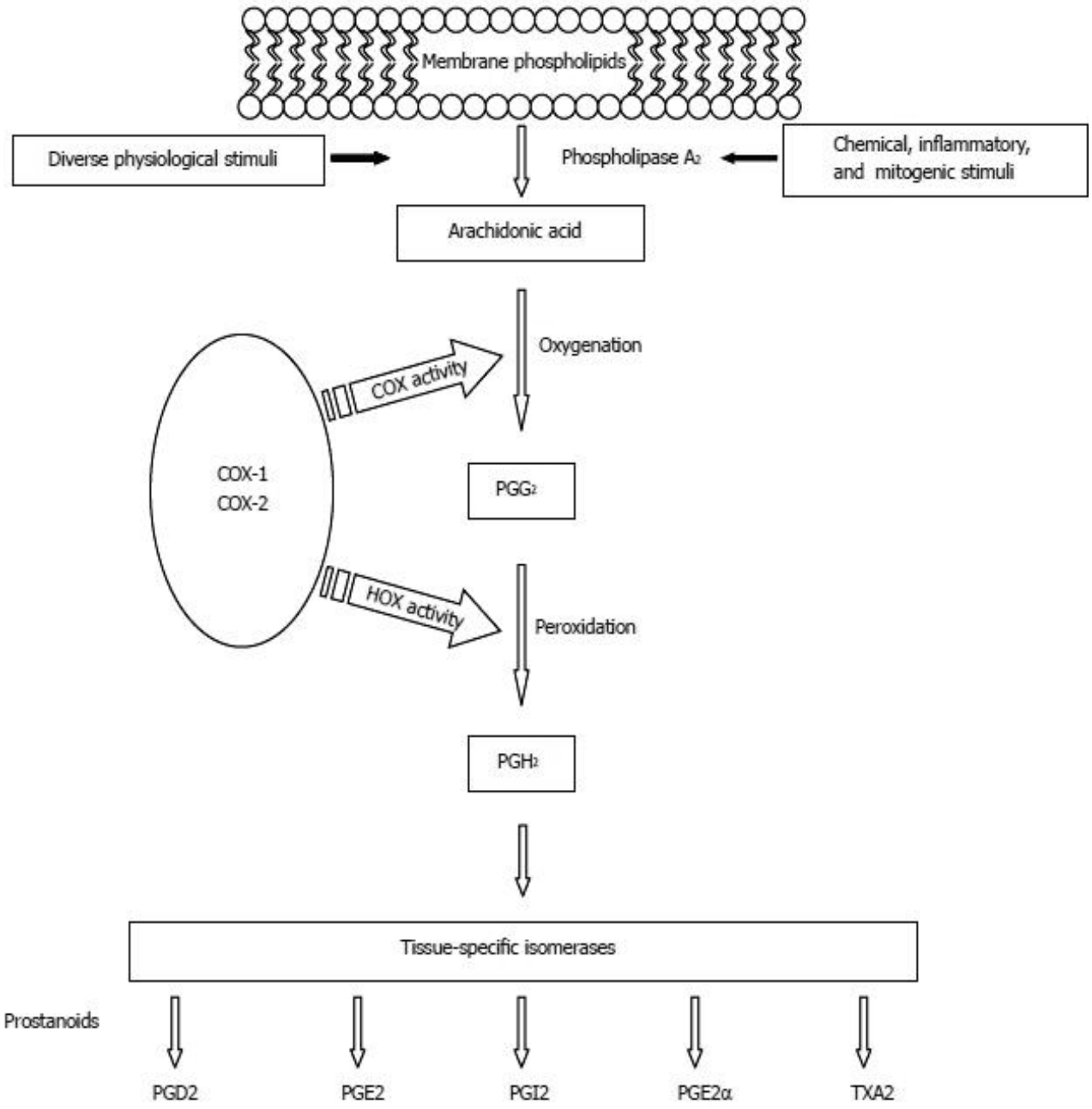


Figure 5. Prostanoids biosynthetic pathway (Cervello et al., 2006).

responses. In humans, prostaglandins are involved in diverse functions, including blood clotting, ovulation, initiation of labor, bone metabolism, nerve growth and development, wound healing, kidney function, blood vessel tone, and immune responses. In contrast to hormones such as cortisone or thyroxin, which have broad systemic effects despite being released from a single site in the body, prostaglandins are synthesized in a broad range of tissue types and serve as autocrine or paracrine mediators to signal changes within the immediate environment (Figure 6) (Dubois et al., 1998).

GENE EXPRESSION OF CYCLOOXYGENASE

The genes of cyclooxygenase-1 and cyclooxygenase-2 are located on chromosomes 9 and 1. The human cyclooxygenase-2 gene is 8.3 kb whereas the cyclooxygenase-1 gene is much larger at 22 kb (Zhab et al., 2004) (Figures 7 and 8).

The cyclooxygenase-1 gene exhibits the features of a housekeeping gene whereas the gene for cyclooxygenase-2 is a primary response gene with many regulatory sites. *In vivo* local increases in cyclooxygenase-2

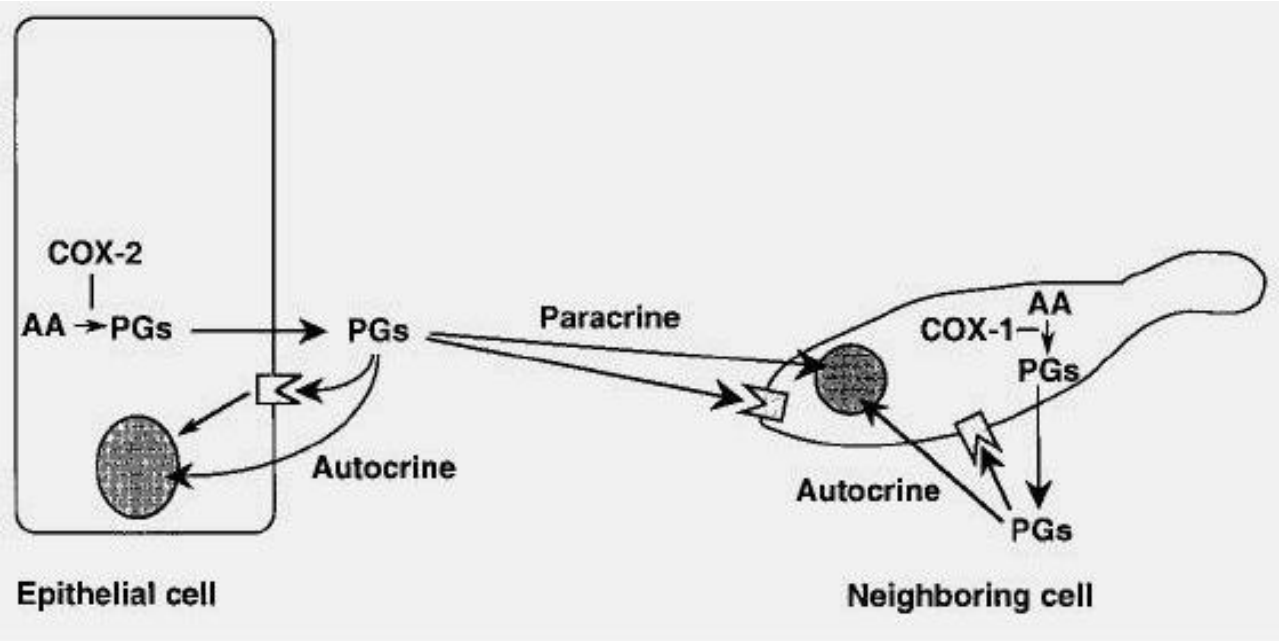
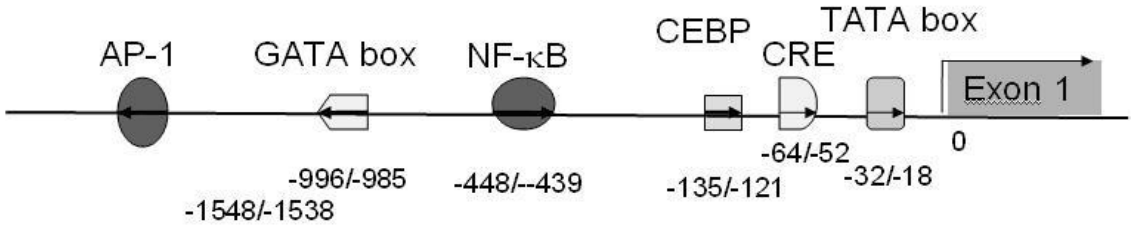


Figure 6. Schematic diagram of potential mechanisms involved in the cyclooxygenase -mediated regulation via paracrine and autocrine pathways (Dubois et al., 1998).



	matrix -matched sequence	synthetic putative binding sequence
AP-1:	GA**TGAC**TAAAA	GAT GGA ATT TTA GTC ATC CGT GTC TCA T
GATA box:	TAGGA**GATA**CTGGT	TAT TTT ACC AGT ATC TCC TAT GAA GGG C
NF-κB:	**GGGG**ATTCCC	GCG GGA GAG GGG ATT CCC TGC GCC
CEBP:	GGCTTAC**GCAA**TTT	CCC ACC GGG CTT ACG CAA TTT T
CRE:	GTCATTT**CGTCA**C	GAA ACA GTC ATT TCG TCA CAT GGG CTT GGT
TATA box:	T**TATA**AAAAGGAAGG	

Figure 7. Structure of human cyclooxygenase-2 gene promoter.

expression have been associated with inflammation, rheumatoid arthritis, seizures and ischemia. The intracellular pathways regulating these events appear numerous and complicated, varying between cell types and cellular stimulus, with nuclear receptors such as peroxisome proliferator-activated receptor-γ attracting more recent attention. There is also regulation of cyclooxygenase-2 expression at the post-translational level, for instance, reactive oxygen species that are implicated in many cancers and are key regulators of cyclooxygenase-2 expression (Timothy et al., 2004).

The regions regulating gene expression of cyclooxy-

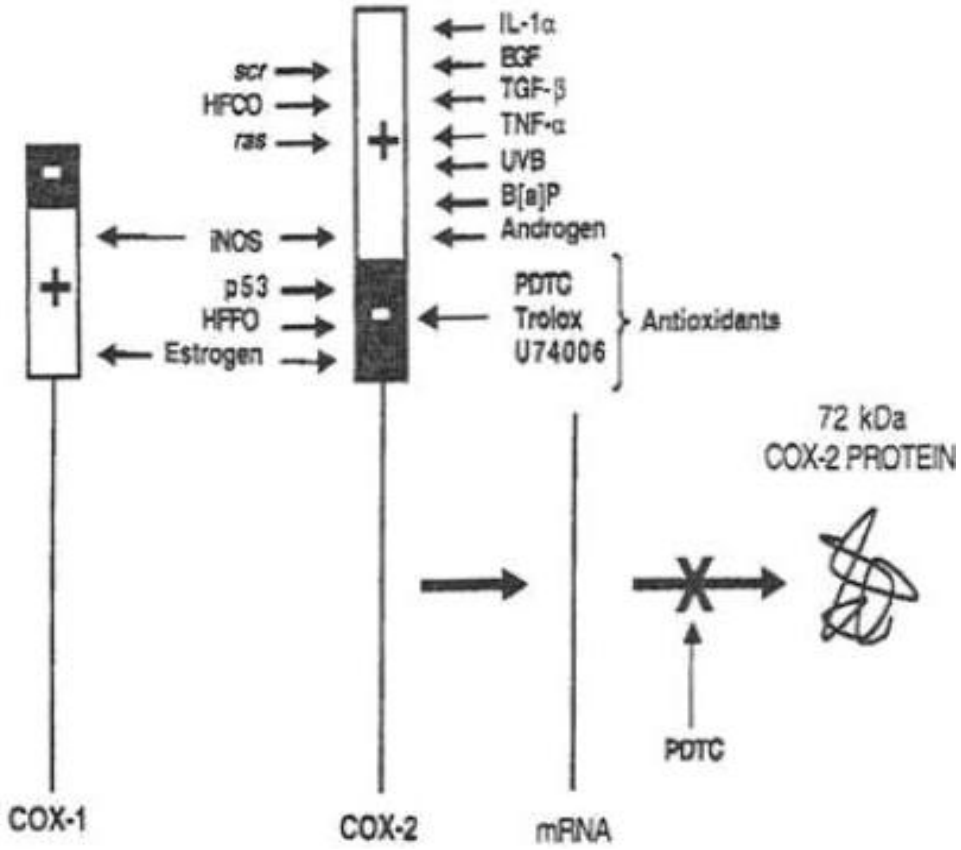


Figure 8. Cyclooxygenase genes (Fossline, 2000).

genase-1 and cyclooxygenase-2 show little similarity. For instance, the promoter and enhancer regions regulating cyclooxygenase-2 contain a variety of response elements that have been shown to explain, at least in part, its inducibility by hormones, growth factors, phorbol esters, cAMP, inflammatory factors, and cytokines. Much less is known about the elements involved in regulating cox-1 gene expression, although studies have reported induction of cyclooxygenase-1 in some circumstances involving differentiation of macrophages. Cyclooxygenase-1 and cyclooxygenase-2 also show major differences in mRNA splicing, stability, and translational efficiency. Regulation of cyclooxygenase-2 at the mRNA level appears to be an important mechanism by which some physiological mediators, notably the corticosteroids (which, consistent with their immunosuppressive properties, downregulate cyclooxygenase-2 expression), act to regulate prostaglandin production (Dubois et al., 1998).

The main inducers of cyclooxygenase-2 are bacterial lipopolysaccharide, pro-inflammatory cytokines-interleukin -1β, interleukin-2 and tumour necrosis factor-α (Zhab et al., 2004).

Of these many stimuli, interleukin-1β has been well known to stimulate cyclooxygenase-2 expression and prostaglandin E2 production in various cell types including monocytes and macrophages, vascular endothelial cells, colon fibroblasts, neuroblastoma cells, and osteoblasts (Kuwano et al., 2004).

Growth factors like epidermal growth factor, platelet derived growth factors and some tumour promoters such as phorbol-12-myristate- 13-acetate also stimulate cyclooxygenase-2 expression. On the other hand, anti-inflammatory molecules such as corticosteroids, interleukin-13, interleukin-10 and interleukin-4 suppress the expression of cyclooxygenase-2 (Zhab et al., 2004).

Cyclooxygenase-2 immunoreactivity is closely related to the differentiation and depth of tumour (Jung et al., 2009). Finally, cyclooxygenase-1 protein, in contrast to cyclooxygenase-2, can also be induced in certain cell types by either phorbol esters or dexamethasone (Zhab et al., 2004).

SIMILARITY AND DIFFERENCE OF CYCLOOXYGENASE AS AN ENZYME

Cyclooxygenase-1 and cyclooxygenase-2: Structural and functional differences

Gene of cyclooxygenase-1, prostaglandin -endoperoxide synthase-1, codes for a 2.8 kb mRNA, which is relatively stable. In contrast, the gene for cyclooxygenase-2,

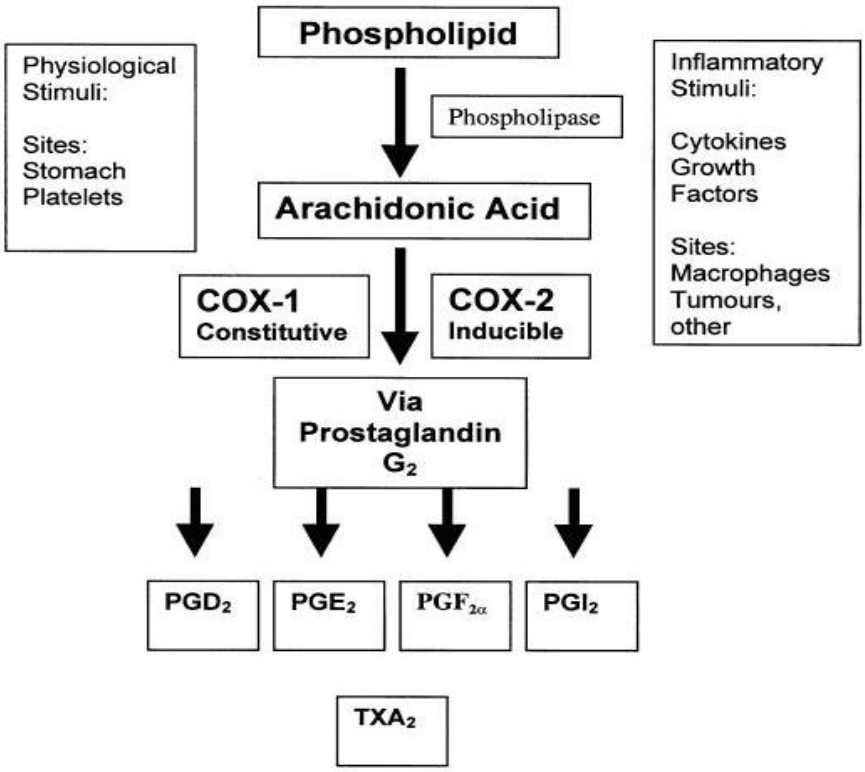


Figure 9. Prostaglandin synthesis via arachidonic acid (Davies et al., 2002).

prostaglandin -endoperoxide synthase -2, is an immediate early gene that is activated by a wide variety of inflammatory and proliferative stimuli, and the 4 kb cyclooxygenase-2 mRNA turns over rapidly due to the presence of instability sequences in the 3'-untranslated region.

Cyclooxygenase-1 provides prostaglandins, which are required for homeostatic functions, including gastric cytoprotection and hemostasis, whereas cyclooxygenase-2 plays the predominant role in prostaglandin formation during pathophysiologic states such as inflammation and tumourigenesis.

The structural and mechanistic bases for the difference in hydrogen peroxide requirement are not fully understood, but site-directed mutagenesis studies indicate that Thr-383, a residue near the heme in cyclooxygenase-2, is at least partially responsible for its greater hydrogen peroxide sensitivity. One more description for the differences in function between two isoforms of cyclooxygenase, cyclooxygenase-2 is capable of metabolizing ester and amide derivatives of arachidonic acid that are poor substrates for cyclooxygenase-1 (Figure 9).

Human cyclooxygenase-1 and cyclooxygenase-2 are homodimers of 576 and 581 amino acids, respectively. Both enzymes contain three high mannose oligosaccharides, one of which facilitates protein folding. A fourth oligosaccharide, present only in cyclooxygenase-2, regulates its degradation. Considering the sixty percent identity in sequence between cyclooxygenase-1 and cyclooxygenase-2, it is not surprising that their three dimensional structures are nearly superimposable. Each subunit of the dimmer consists of three domains, the epidermal growth factor domain, the membrane binding domain, and the catalytic domain comprising the bulk of the protein, which contains the cyclooxygenase and peroxidase active sites on either side of the heme prosthetic group. Cyclooxygenase-2 has a dual role in inflammation, initially inducing the inflammatory process and later aiding in its resolution (Rouzer et al., 2007).

Spatial structure of cyclooxygenase enzymes

i. Amino –terminal signal peptide
ii. Dimerization domain
iii. Membrane binding domain
iv. Catalytic domain

CYCLOOXYGENASE INHIBITORS

Nonsteroidal anti-inflammatory drugs have been used clinically for more than 100 years. Cyclooxygenase-2 selective inhibitor is a form of non-steroidal anti-

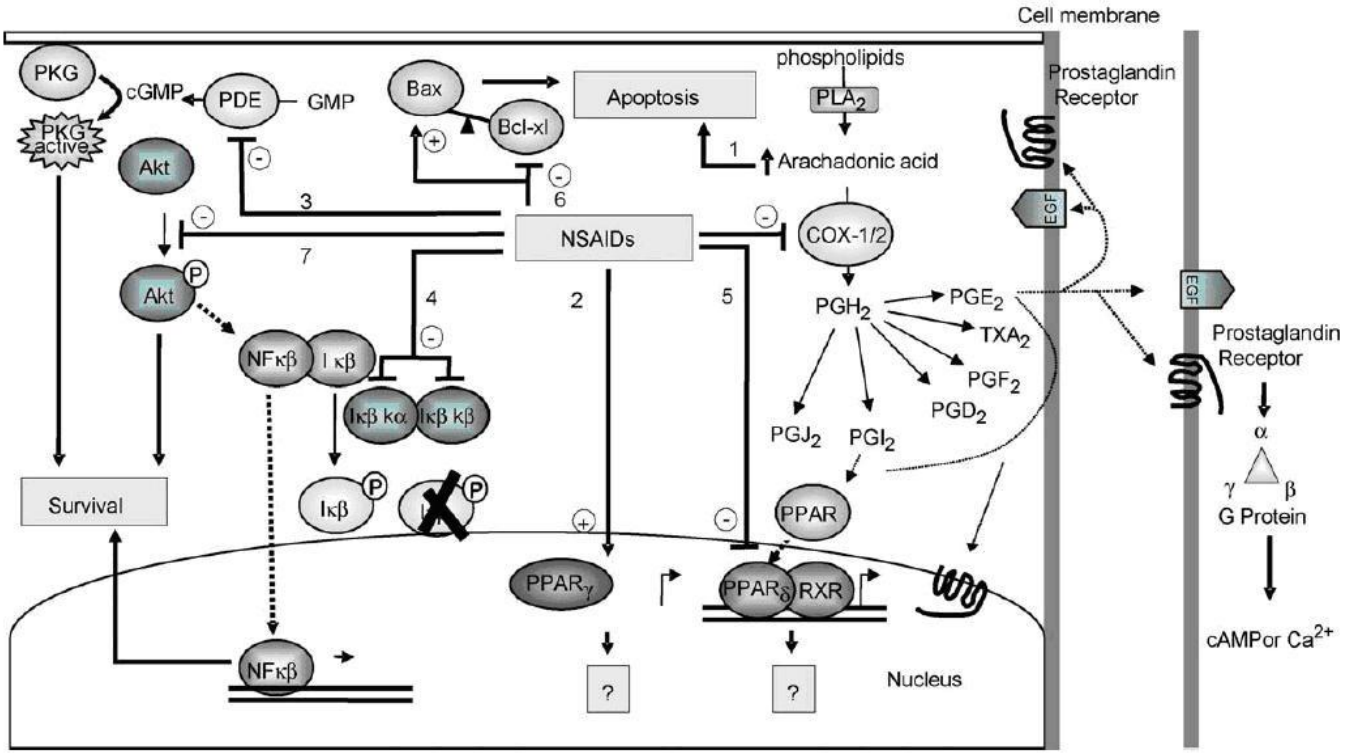


Figure 10. Molecular mechanisms for cyclooxygenase-2 and NSAIDs (Zhab et al., 2004).

inflammatory drug that directly targets cyclooxygenase-2, an enzyme responsible for inflammation and pain (Figure 10).

Selectivity for cyclooxygenase-2 reduces the risk of peptic ulceration, and is the main feature of celecoxib, rofecoxib and other members of this drug class. Cyclooxygenase-2 selectivity does not seem to affect other adverse effects of nonsteroidal anti-inflammatory drugs, and some results have aroused the suspicion that there might be an increase in the risk for heart attack, thrombosis and stroke by a relative increase in thromboxane. Rofecoxib was taken off the market in 2004 because of these concerns. Drug classes and their drugs members are shown as follows: cyclooxygenase-1 inhibitors include ketorolac, flurbiprofen, ketoprofen, and piroxicam; cyclooxygenase-2 inhibitors include sulindac, nimesulid etodolac, meloxicam, celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib and valdecoxib; and inhibitors between cyclooxygenase-1 and 2 includes asprin, naproxen, indomethacin and ibuprofen (Figure 11).

CYCLOOXYGENASE-2 IN ANGIOGENESIS

Angiogenesis is an important factor in tumor development. Tumor associated angiogenesis is mediated by the migration and proliferation of host endothelial cells and is a requisite for tumor growth. The mechanisms for promotion of tumor-associated angiogenesis are suggested to be activated in the early stages of tumor development. Vascular endothelial growth factor, TGFα and β, FGF and chemokines like IL-8 are implicated in tumor-related angiogenesis in lung cancer (Figure 12).

Because cyclooxygenase-2 has been reported to be localized in both tumor cells and the adjacent stromal cells, prostaglandins generated through the presence of cyclooxygenase-2 might act on the tumor cells or surrounding stroma cells to facilitate tumor development (Patel and Chiplunkar, 2007).

CYCLOOXYGENASE AND PROLIFERATION

Selective inhibition of cyclooxygenase-2 in these model systems results in a decrease in cell rapid reproduction and restoration of the apoptotic rate. The role of cyclooxygenase-2 in the production and development of neoplasic tumours is well established, especially in colon cancer. Moreover, several authors have reported that increased tissue cyclooxygenase-2 expression and prostaglandin production may contribute to the development of other cancers, including hepatocellular carcinoma (Kurt et al., 2003).

Enhanced cyclooxygenase-2 induced synthesis of prostaglandins stimulates cancer cell rapid reproduction, and increases metastatic potential.

Cyclooxygenase-2 is also closely involved in the carcinogenesis process (Figure 13) and is over expres-

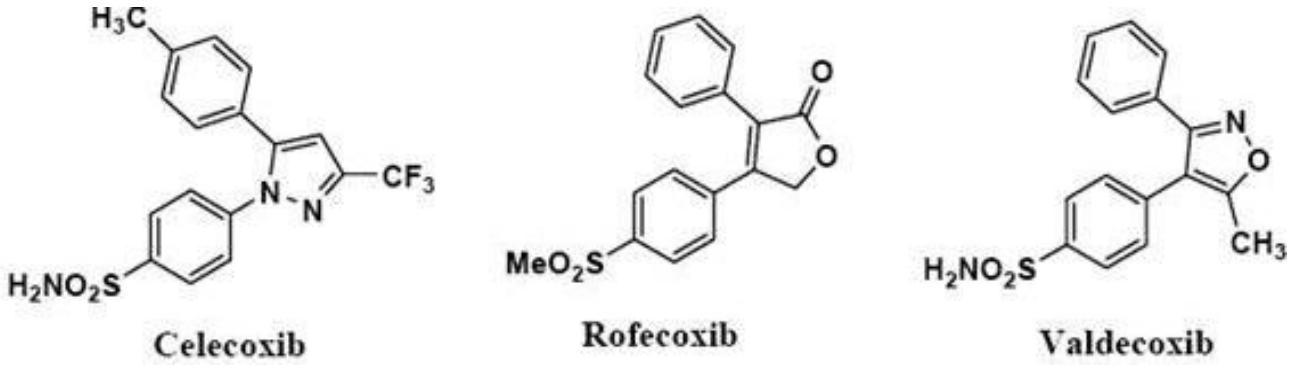


Figure 11. Structure of specially inhibitor of cyclooxygenase (Praveen et al., 2003).

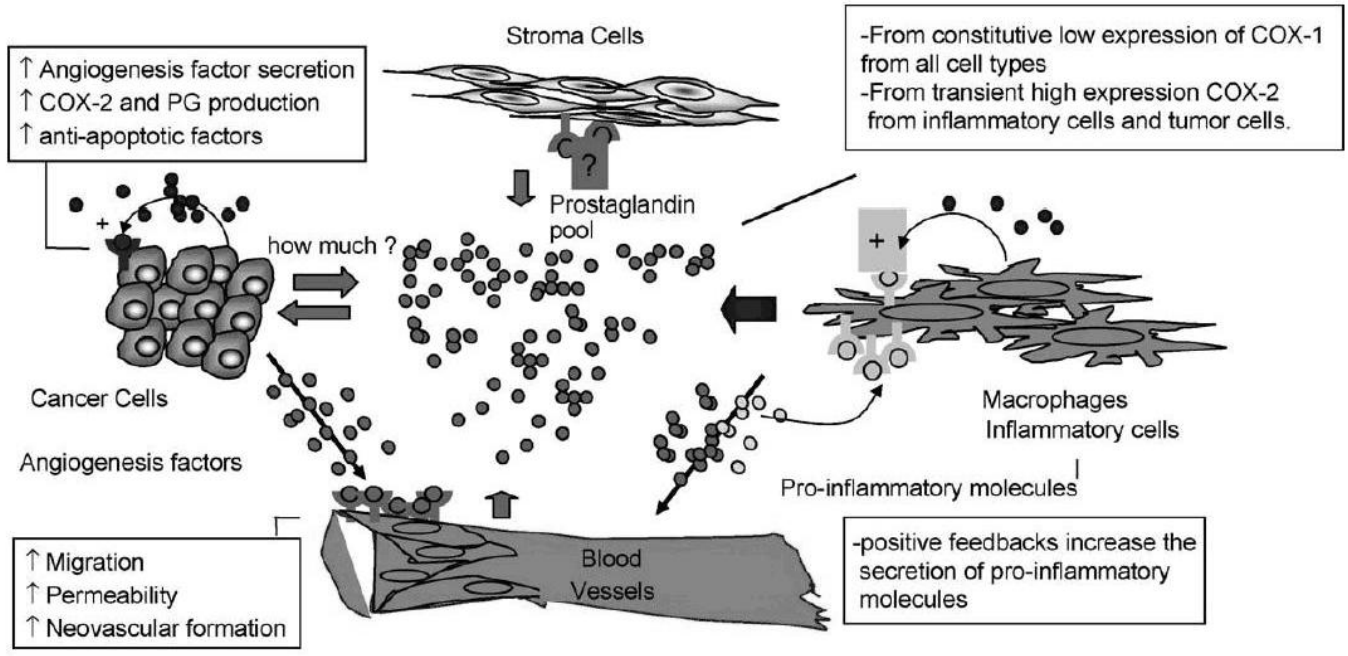


Figure 12. Cyclooxygenase-2 in angiogenesis (Zhab et al., 2004).

sed in adenocarcinoma in comparison with no cancerous mucosal regions in colon cancers and gastric cancers. Cyclooxygenase-2 plays an important role in the carcinogenesis and development of many kinds of human cancers such as colorectal cancer, gastric cancer, lung cancer and esophageal cancer. Most of the colorectal cancers are derived from adenomas. Elevated levels of mRNA and protein of cyclooxygenase-2 are known to be associated with esophageal, head and neck, breast, lung, prostate, and other cancers, indicating a close involvement of cyclooxygenase-2 in tumour progression and other pathological phenotypes in various malignant tumours (Kuwano et al., 2004).

Work in cell culture models has shown that cyclooxygenase-2 expression contributes significantly to the tumourigenic potential of epithelial cells by increasing adhesion to extra cellular matrix and making them resistant to apoptosis. Recent researches indicate that cyclooxygenase may play a vital role in the regulation of angiogenesis associated with neoplastic tumour cells. Hence, cyclooxygenase inhibitors may block the growth of blood vessels into developing tumours. Genetic evidence supporting a role for cyclooxygenase-2 in the development of intestinal neoplasia has also been reported (Dubois et al., 1998).

Transgenic mice, over expressing cyclooxygenase-2 in mammary glands develop mammary gland hyperplasia, dysplasia and metastatic tumours. Over expression of cyclooxygenase-2 under a keratin-5 promoter, causes transitional cell hyperplasia and carcinoma in urinary

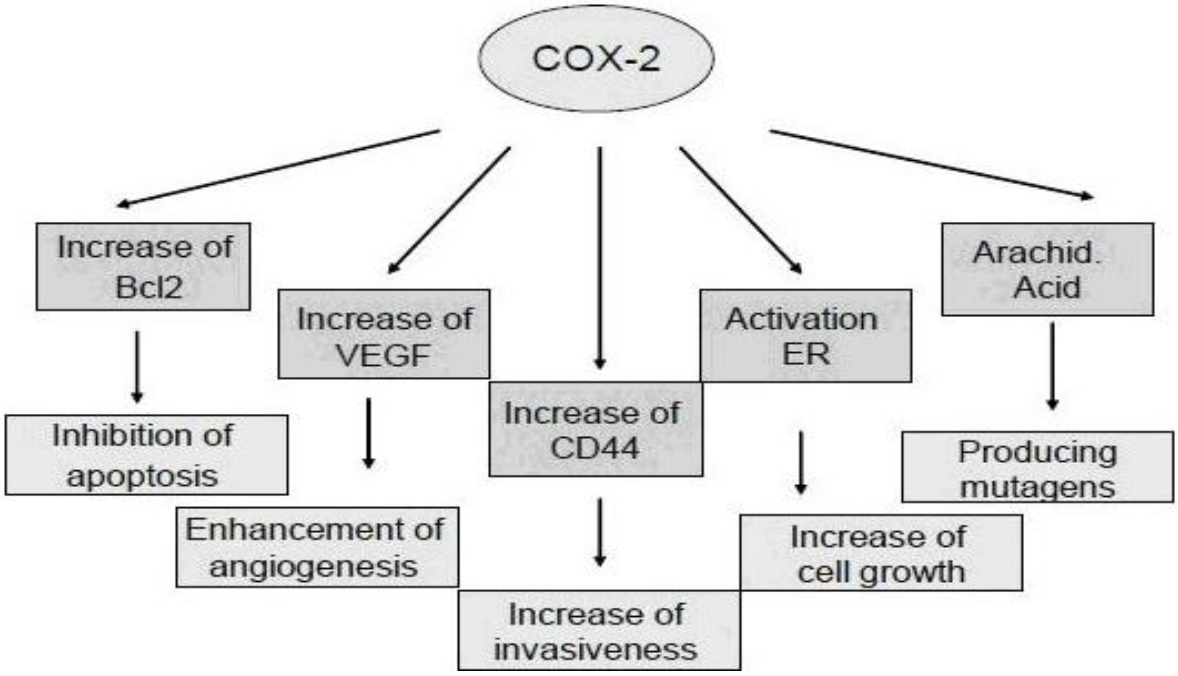


Figure 13. Contribution of cyclooxygenase-2 to the carcinogenesis (Krcova et al., 2008).

bladders of the transgenic mice. In addition, over expression of cyclooxygenase-2 *in vitro* promotes cell rapid reproduction in human prostate cancer cells and enhances invasiveness of human bladder, breast and colon cancer cells. However, the function of cyclooxygenase-2 in osteosarcoma is unclear.

Selective cyclooxygenase-2 inhibitors have been reported to inhibit osteosarcoma cell rapid reproduction and invasion *in vitro* and tumour growth and metastasis *in vivo*. However, there is no direct evidence elucidating the function of cyclooxygenase-2 in osteosarcoma because cyclooxygenase-2 inhibitors may affect other cellular molecules. Moreover, a recent report that murine cyclooxygenase-2 over expression in osteosarcoma cells inhibited cell growth via inducing cell cycle arrest and apoptosis raise a question about the function of cyclooxygenase-2 in osteosarcoma (Figure 14).

Work in Inhibition of cyclooxygenase-2 and inducible nitric oxide synthase by silymarin in proliferating mesenchymal stem cells has shown that COX-2 and iNOS that affect cell proliferation are regulated by glutathione level. Contribution of factors involoved such as COX-2, iNOS, glutathione and H2O2 would suggest the mechanisms by which MSC proliferation is stimulited. The effect of COX-2 induction on cell proliferation depends on several factors, including the pattern of prostanoid production and the coupling of cAMP to growth inhibitory/growth promoting pathways (Ahmadi-Ashtiani et al.,2011).

In another research, U2OS human osteosarcoma cells were transfected with cyclooxygenase-2, and it was discovered that cyclooxygenase-2 over expression increased cell rapid reproduction rates, mobility, and invasiveness (Lee et al., 2007).

The inner cell mass of blastocysts possesses apoptotic machinery and antiapoptotic defense program. Cultured mouse embryonic stem cells derived from inner cell mass undergo apoptosis when subjected to prolonged hypoxia or methylglyoxal-induced oxidative stress.

Prostacyclin or PG I2 and PG E2 have emerged as important endogenous molecules for protection of somatic cell survival. Endogenously produced PG I2 has been shown to protect endothelial cells from H_2O_2-induced apoptosis and renal interstitial cells from hyper tonicity-induced apoptosis via the peroxisome proliferators-activated receptors δ pathway. PG E2 has been reported to protect dendritic and neuronal cells from apoptosis via PG E2 receptors EP2 and EP4. PG I2 and PG E2 are synthesized in diverse somatic cells via the cyclooxygenase pathway. Cyclooxygenase-2 derived PG E2 protects mouse embryonic stem cells form H_2O_2-induced apoptosis via EP2 mediated activation of phosphatidylinositide-3 kinase to Akt (that is, member of the non-specific serine/threonine-protein kinase family) pathway. PG E2 was reported to protect against apoptosis in several cell types including bone marrow-derived and monocyte-derived dendritic cells, neurons, epithelial cells and colon cancer cells. However, PG E2 was also reported to induce apoptosis in articular chondrocytes and hippocampal neurons. The reason for the opposite actions of PG E2 is unclear. It may be due to

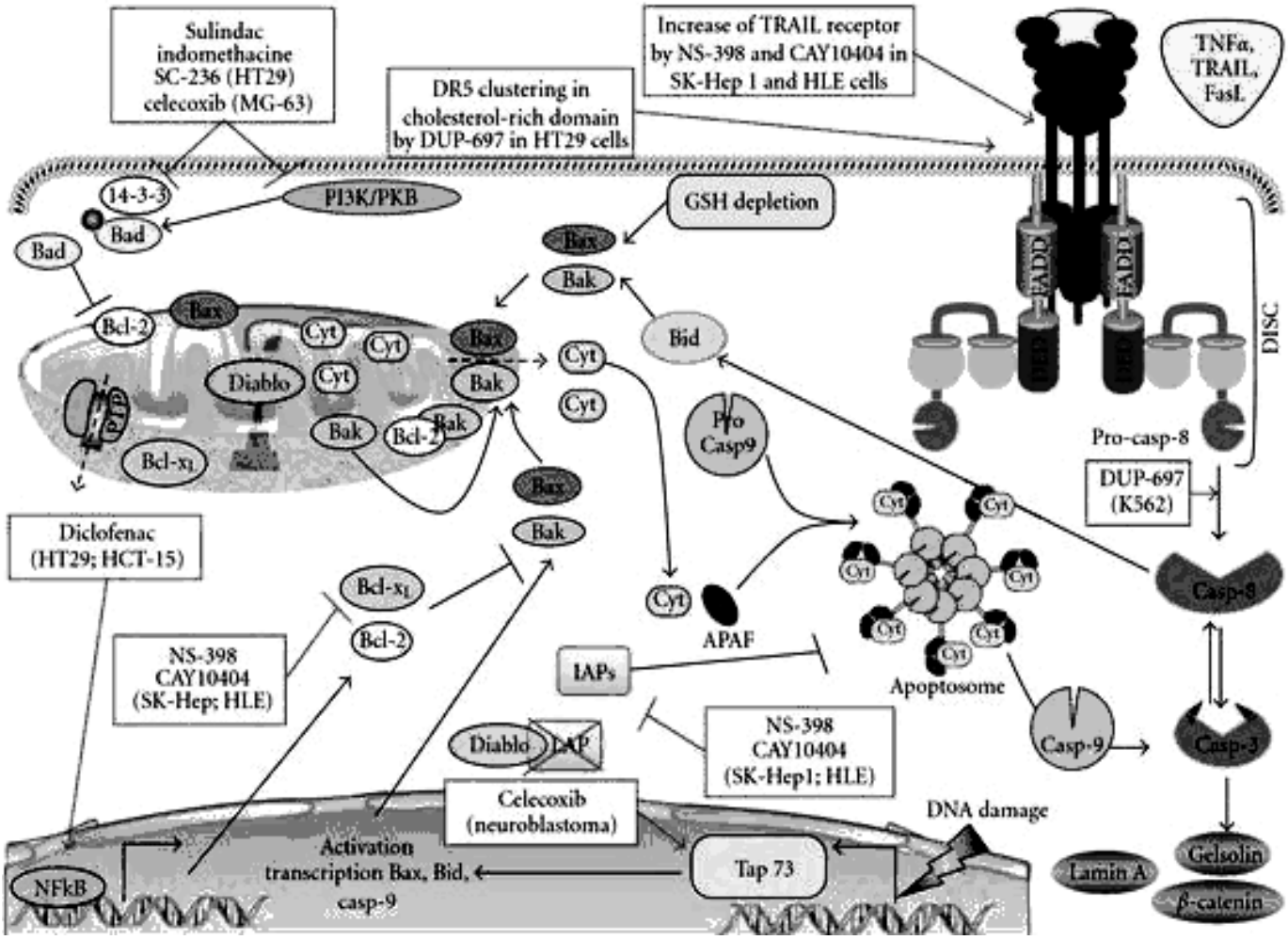


Figure 14. Effects of cyclooxygenase-2 inhibitors on apoptosis.

different cell types or different apoptotic stimuli and may reflect the actions of different EP receptor isoforms. In most differentiated somatic cells, cyclooxygenase-1 is constitutively expressed while cyclooxygenase-2 is expressed after stimulation with diverse agents including cytokines, growth factors and endotoxins like lipopolysaccharide. Cyclooxygenase-2 is expressed in large abundance in hematopoietic progenitor cells, mesenchymal stem cells and cancer cells. Constitutive expression of cyclooxygenase-2 in mouse embryonic stem cells may reflect its important physiological role in stem cells. One key role is to enable stem cells to resist damage by oxidative stress, serum depletion and other cytotoxic conditions and maintain their unique properties of self renewal and differentiation (Liou et al., 2007). Increase in PG E2 activity produces factors like:

i. Increase of B-cell lymphoma 2 causes inhibition of apoptosis. B-cell lymphoma 2 inhibits cytochrome c, caspase 9, caspase 3 and finally apoptosis (Sun et al., 2002).

ii. Activity of phosphatidylinositol 3-kinases stimulates rapid reproduction (Lee et al., 2007).

iii. Increase in matrix metalloproteinase-2 and matrix metalloproteinase-9 causes metastasis (Figures 15 and 16) (Lee et al., 2007).

CYCLOOXYGENASE AND DIFFERENTIATION

Both cyclooxygenase-1 and cyclooxygenase-2 have significant roles in regulating epidermal differentiation (Tiano et al., 2002). Cyclooxygenase-2 selective inhibitors are also able to induce cell differentiation and cell apoptosis (Mehmet et al., 2008).

In vivo studies have more definitively indicated a role for both cyclooxygenase isoforms in keratinocyte differentiation, as the genetic deletion of either cyclooxygenase-1 or cyclooxygenase-2 leads to premature terminal differentiation of epidermal keratinocytes (Jacqueline et al.,

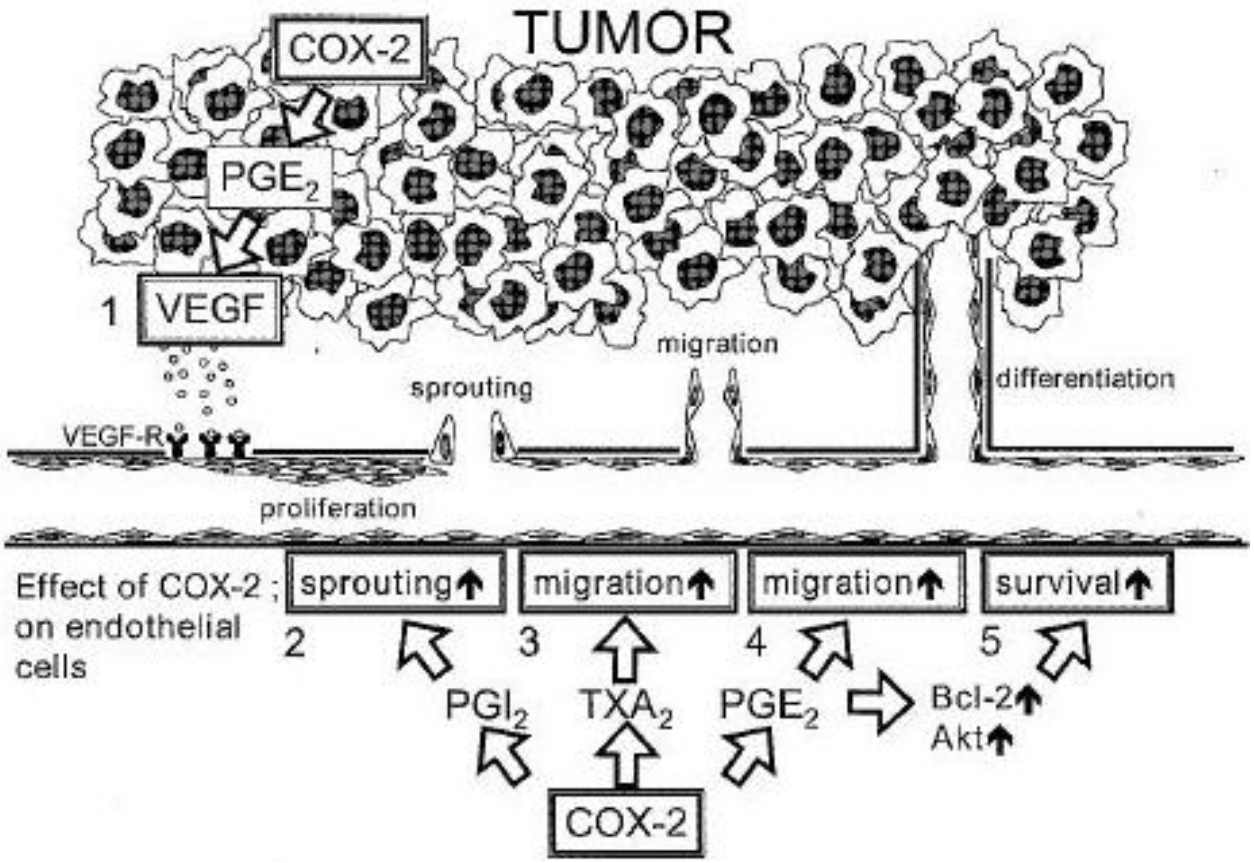


Figure 15. Cyclooxygenase-2 induces tumor angiogenesis via multiple pathways (Ohno et al., 2005).

2003). Skeletal muscle satellite cells are myogenic stem cells found in adult skeletal muscle. They play important roles in muscle repair and hypertrophy. Satellite cells normally remain in a quiescent state between the muscle-fiber plasma membrane and basal lamina. Following trauma to the muscle fiber, satellite cells become active and move along the length of the fiber to the site of injury, where they undergo proliferation, differentiation, and fusion. Some of the cells generated by the proliferative process eventually become part of the mature muscle fiber, whereas others return to the quiescent satellite cell pool. The subsequent differentiation of satellite cells involves the expression of a family of myogenic regulatory factors that promote expression of muscle specific proteins (Xinping et al., 2002). One member of the myogenic regulatory factor family normally occurs as satellite cells withdraw from the cell cycle and differentiate into myocytes, in preparation for fusion. *In vivo*, differentiated satellite cells fuse with damaged muscle fibers to repair areas of damage. Cyclooxygenase-1 and cyclooxygenase-2 have main role in regulating satellite cell rapid reproduction. Inhibition of cyclooxygenase-1 and cyclooxygenase-2 has effect on satellite cell proliferation, differentiation, and fusion (Mendias, 2004). PG E2 produced locally may act during nuclear events and this action may be differentiation-specific in adipocytes. The intracellular localization of lipocalin-type PG D2 synthase was also altered during adipogenesis. In pre-adipocytes, lipocalin-type PG D2 synthase was restricted to the perinuclear- golgi region of the cell. Prostaglandins play a pleiotropic role in differentiation events *in vitro*. The findings of differential cyclooxygenase-1, cyclooxygenase - 2 and PG synthase isoforms expression, as well as changes in intracellular distribution of the proteins, suggest that the arachidonic acid cascade is under complex control during adipogenesis (Xie et al., 2006). Both cyclooxygenase-1 and cyclooxygenase-2 have been recognized in osteoblasts; the differential roles of the two cyclooxygenases in bone formation remain unclear. Mice lacking cyclooxygenase-2, but not cyclooxygenase-1 expression, display reduced bone resorption in response to parathyroid hormone or 1, 25-hydroxyl vitamin D3. Cyclooxygenase-2 may also have a role in bone formation. Systemic or local injection of PG E2 stimulates bone formation. The levels of prostaglandins E and F are increased between days 3 and 14 in tissues obtained from rabbit tibia fractures, which means that the metabolites of cyclooxygenase activity have long been suspected to have a role in skeletal reparative processes. Some genes are specifically required for osteoblast differen-

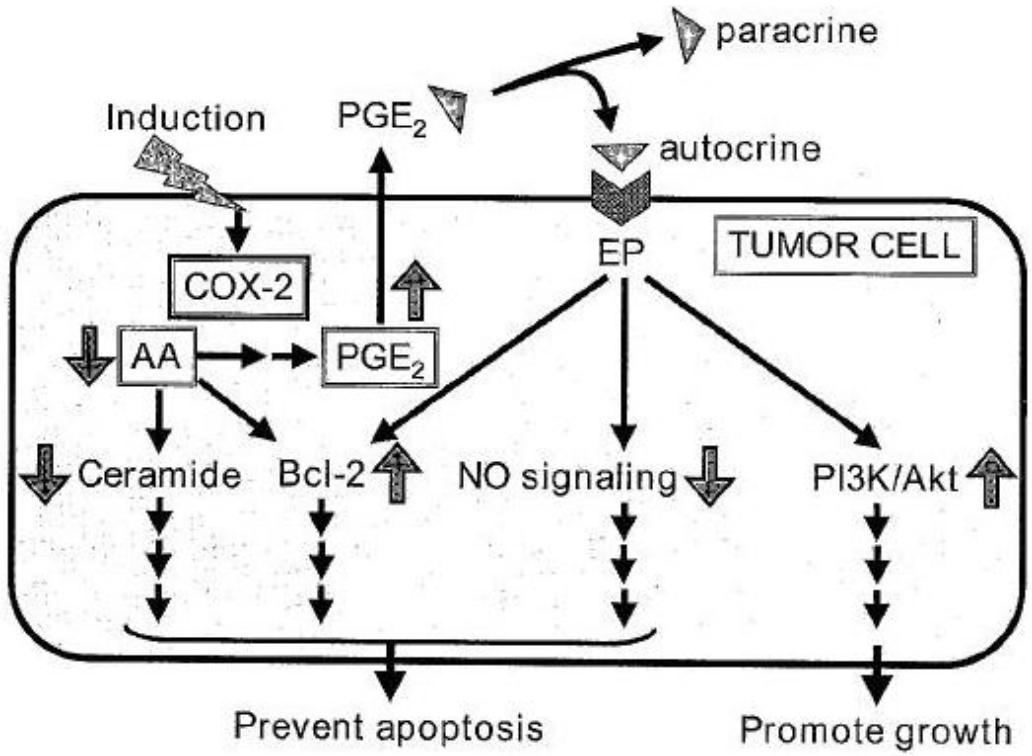


Figure 16. Cyclooxygenase-2 mediates the prevention of cell apoptosis and promotion of cell growth (Ohno et al., 2005).

tiation and bone formation (Figure 17). Two of these genes are *cbfa1* and *osterix.*

Under basal conditions, cyclooxygenase-2 activity maintains a population of mesenchymal stem cells in a pre osteoblast state responsive to additional osteoblastic signals. During injury, elevated cyclooxygenase-2 expression increases the osteoblastic potential of mesenchymal stem cells and supports their differentiation to osteoblasts in response to osteogenic signals (Xinping et al., 2002). Metalloproteinase-9, β2 integrin CD11c, tissue factor, thrombomodulin, tumour necrosis factor α, and phospholipase A, are all increased or triggered during monocyte differentiation into macrophages (Figure 18). Macrophages, once activated by inflammatory stimuli, synthesize and release eicosanoids that are potent modulators of inflammation. The rate limiting step in this event is represented by the cyclooxygenases. Differentiation of macrophages into monocyte needs cyclooxygenas-2 (Barbieri et al., 2003; Stachowsk et al., 2005).

Activation of PKC, tyrosine kinase and of the mitogen-activated protein-kinase extra cellular signal-regulated kinases 1 and 2 was essential for cyclooxygenase-2 induction during differentiation. Reactive oxygen species generators per se, trigger cyclooxygenase-2 protein and mRNA in monocytes. The nicotinamide adenine dinucleotide phosphate oxidase subunit $p47^{phox}$ is up regulated during monocytic differentiation and this event is causally linked to cyclooxygenase-2 expression. Nicotinamide adenine dinucleotide phosphate oxidase inhibitors markedly prevented cyclooxygenase-2 protein and mRNA triggered by differentiation. Reactive oxygen species are ubiquitous, highly diffusible and reactive molecules produced by reduction of molecular oxygen, and they include species such as super oxide anion, hydrogen peroxide and hydroxyl radical. They regulate, however, multiple cellular functions such as growth and differentiation, rapid reproduction, apoptosis and gene expression, acting through both transductional and transcriptional pathways (Akunda et al., 2003).

The intracellular sources contributing to reactive oxygen species generation in monocytes are several, and include cyclooxygenases, lipoxygenases, mitochondrial respiration and nicotinamide adenine dinucleotide phosphate oxidase. Nuclear factor kappa-light-chain-enhancer of activated B cells activation is increased by reactive oxygen species generators whereas nicotinamide adenine dinucleotide phosphate oxidase inhibitors prevent it. Besides nuclear factor kappa-light-chain-enhancer of activated B cells, extra cellular signal-regulated kinases 1 and 2, but not p38 activation, is critically involved in cyclooxygenase-2 induction during monocytic differentiation. Activation of Rac2, a GTPase of the Rac protein family, is required for full activation of nicotinamide adenine dinucleotide phosphate oxidase. These results indicate that Rac2 activity is cardinal to the expression of cyclooxygenase-2 during monocyte differentiation

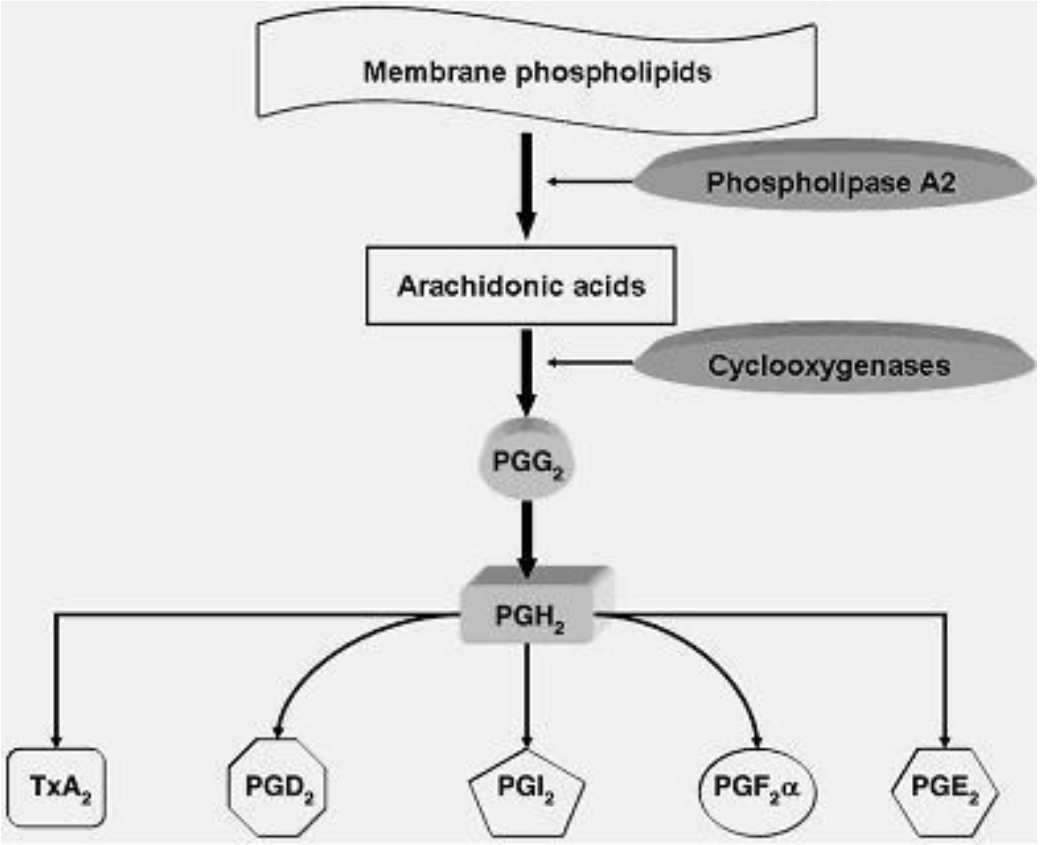


Figure 17. Metabolic products of cyclooxygenase-2 (Mehmet et al., 2008).

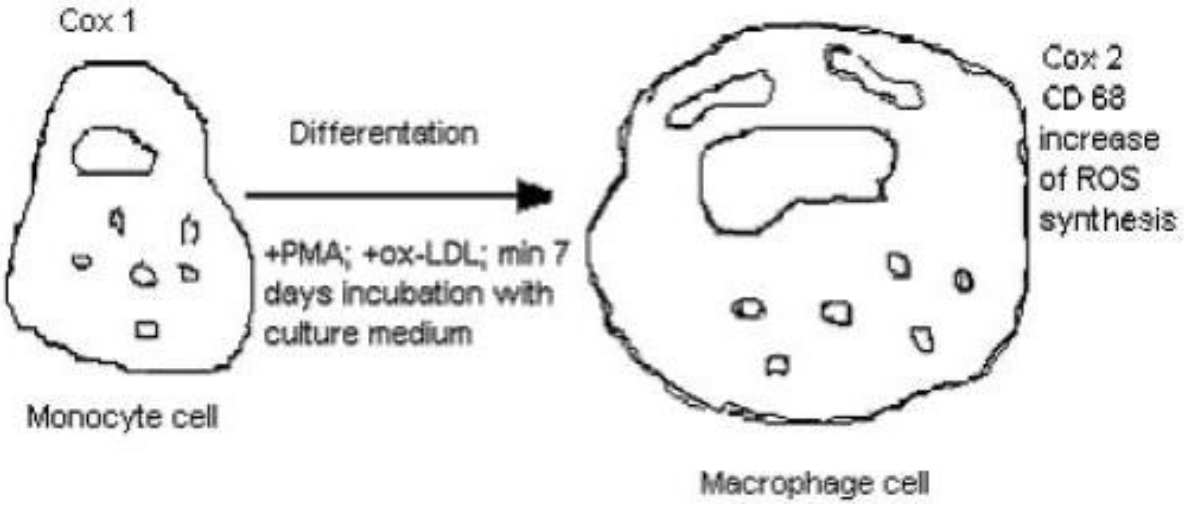


Figure 18. Diagram of monocyte differentiation into a macrophage (Stachowsk et al., 2005).

(Barbieri et al., 2003). Diosgenin [(25R)-5-spirosten-3b-ol] is a steroidal saponin that can be found in several plant species induced by megakaryocytic differentiation of HEL cells through sustained activation of extra cellular signal-regulated kinases and inhibition of p38 mitogen-activated protein kinase pathways. Cyclooxygenase-2 and thromboxane synthase activities were involved in megakaryocytic differentiation induced by diosgenin in HEL cells. Prostanoids are a cluster of bioreactive lipids that interact with specific cell membrane receptors of the G protein

coupled superfamily, playing important roles in many cellular responses and physiological processes. They are generated through cyclooxygenase and peroxydase activities.

Membrane-associated PG E synthase, cyclooxygenase-2, cyclooxygenase-1, and thromboxane synthase existed in terminally differentiated megakaryocytic HEL cells and demonstrated that cyclooxygenase-2, membrane-associated PG E synthase-1, and thromboxane synthase, but not cyclooxygenase-1, were induced during diosgenin-induced megakaryocytic differentiation. Diosgenin -differentiated cells showed nuclear polyploidization and increased expression of platelet marker CD41 associated with a decrease in GpA and NS-398 or BM 567 pretreat-ment which decreased megakaryocytic differentiation induced by diosgenin in HEL cells (Cailleteau et al., 2008). In hepatocellular carcinoma, the expression pattern of cyclooxygenase-2 protein is well correlated with the differentiation grade, suggesting that abnormal cyclooxygenase-2 expression plays an important role in hepatocarcinogenesis while inhibition of cyclooxygenase-2 can induce growth suppression of hepatoma cell lines via induction of apoptosis (Huang et al., 2005). Dedifferentiation of chondrocytes caused significant increases in cyclooxygenase-2 expression and PG E2 production. Epidermal growth factor -induced dedifferentiation was caused by epidermal growth factor receptor-mediated activation of extra cellular signal-regulated protein kinases 1 and 2 but not p38 kinase, whereas the activation of both extra cellular signal-regulated kinases 1 and 2 and p38 kinase was necessary for cyclooxygenase-2 expression and PG E2 production. Epidermal growth factor -induced cyclooxygenase-2 expression and PG E2 production is regulated by extra cellular signal-regulated kinases 1 and 2 and p38 kinase signaling. Epidermal growth factor in articular chondrocytes stimulates cyclooxygenase-2 expression and PG E2 production via extra cellular signal-regulated kinases and p38 kinase signaling in association with differentiation status (Yun-Hyun et al., 2002). Over expression of p38 mitogen-activated protein kinase induces a cox-2 reporter, whereas over expression of dominant negative p38 mitogen-activated protein kinase represses interleukin-1β-induced promoter expression. Differentiated articular chondrocytes are highly responsive to interleukin-1β and p38 mitogen-activated protein kinase mediates this response by inducing cyclooxygenase-2 gene expression (Sylvie et al., 2002).

CONCLUSION

Over expressed cyclooxygenase-1 or cyclooxygenase-2, a role for cyclooxygenase enzymes in cell rapid growth factor, mitogen and cytokine stimuli, has been ascertained. Selective inhibition of cyclooxygenase-2 in these model systems results in a decrease in cell rapid reproduction and restoration of the apoptotic rate (Kurt et al., 2003). Cyclooxygenase-2 expression contributes significantly to the tumorigenic potential of epithelial cells by increasing adhesion to extra cellular matrix and mak-ing them resistant to apoptosis (Dubois et al., 1998). Cyclooxygenase-2 is consistently over expressed in a large percentage and variety of human and rodent tu-mors. At the cellular level, cyclooxygenase inhibitors have been shown to inhibit proliferation, induce apopto-sis, inhibit angiogenesis, reduce carcinogen activation, and stimulate the immune system. An important conside-ration is the potential consequences of inhibition of cyclo-oxygenase-2 in tissues where this enzyme has been constitutively expressed. Whether or not selective inhibi-tion of cyclooxygenase-2 fulfills the therapeutic potential will depend on long-term safety of selective cyclooxy-genase -2 inhibitors (Gupta et al., 2011).

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