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Review Article

CYCLOOXYGENASE -2 AND CANCER: A silent link with fatal ending.

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Abstract: Remarkable evolutions in pathogenic role of Cyclooxygenase-2 (COX-2) in tumorigenesis have diverted the cancer research in the direction of COX-2 inhibitors. An expanding body of evidence indicates that down regulation of cyclooxygenase 2 will be an important strategy for preventing cancer because COX-2 catalyzes the formation of prostaglandins(PGs) and these prostaglandins have multiple effects that favor tumorigenesis. It is proved that during the process of chronic inflammation several cytokines and inflammatory mediators are released which act during the different stages of carcinogenesis. Among all these mediators COX 2 are the key player. COX2 is found to be over expressed in several malignancies like colorectal carcinoma, squamous cell carcinomas of head and neck, carcinomas of urinary bladder, liver, stomach, breast as well as hematological malignancies. Our review article aims to summarize the role of COX 2 in various cancers and the mechanisms responsible for carcinogenesis. The informations gathered in this review are taken mainly from articles published in different international journals.

Key Words: COX-2 Cyclooxygenase 2.

Inflammation And Cancer: An Ancient Link

The possible role of chronic inflammation in tumorigenesis was first described 150 years ago. As early as 1863, Virchow indicated that cancer tends to occur at sites of chronic inflammation because prolonged irritation, tissue injury and activated local host response ultimately favored cell proliferation[1,16,17,18]. Yet, it is only during the last decade that clear evidence has been obtained that inflammation plays a critical role in carcinogenesis[16]. It is also proved that cancer risk is positively associated with the severity and duration of inflammatory disease. Inflammation is the major reaction of natural immunity to provide resistance against pathogens. It can be induced by bacterial, viral, parasitic infections as well as physical and chemical injuries[2]. The association between inflammation and cancer has been described by several epidemiologic and clinical trials[1,3]. For example, the risk of colorectal carcinoma is 10 fold greater if associated with ulcerative colitis or crohn's disease[4,5]. Likewise dysplastic progression leading to nasopharyngeal carcinoma is caused by Epstein Barr Virus(EBV)[3,6]. Helicobacter pylori infection is associated with adenocarcinoma of stomach[3,6]. Cholangiocarcinoma of bile tract is caused by Clonorchis sinensis infection[6]. HBV and HCV are the leading cause of hepatocellular carcinoma[6]. Human papilloma virus infection is the leading cause of penile and anogenital carcinoma. Schistosomiasis and human herpes virus type 8 may cause urinary bladder cancer and Kaposi's sarcoma respectively[1,7]. Moreover chronic inflammation caused by etiologic agents other than infection may also cause cancer. For example, Cancers of esophagus, pancrease and gall bladder may be caused by inflammatory diseases such as esophagitis, Barrett's metaplasia, and chronic pancreatitis[6,8]. Possible link is found in Marjolin's ulcer and skin cancer, asbestos and mesothelioma, silica and cigarette smoke and bronchial cancer(6), chronic asthma and lung cancer[9,10,11]. All these evidences clearly indicate that chronic inflammation plays a key role in the development and modulation of different steps of carcinogenesis. It can initiate tumor by triggering the production of reactive oxygen species(ROS), responsible for DNA damage thus increasing the rate of mutations[12]. It may also be concerned with tumor promotion, where inflammation triggers the secretion of growth factors, such as epithelial(EGF) and fibroblast growth factor(FGF). These, in turn favor the proliferation of initiated tumor cells by determining an imbalance cell proliferation and cell death stimuli due to the activation of different cell survival pathways[13,14,62,65]. It is well established that different cytokines (TNF-α, IL1β, IL 6 and IL 8) produced during inflammation can also activate several survival pathways which prevent tumor cells from cell death. One such example is TNF-α which is produced by tumor and immune cells and leads to the survival of

cancer cells by the up regulation of antiapoptotic proteins, i.e, Bcl-2 via the activation of the nuclear factor kappa B (NFκB) [15,63,64]. Amongst the different mediators of inflammation, the cyclooxygenase-2 is clearly implicated in cancer as shown in **fig1**. This review focuses on COX-2 an inducible isoform and intends to evaluate and summarize different studies regarding link between COX-2 and various cancer.

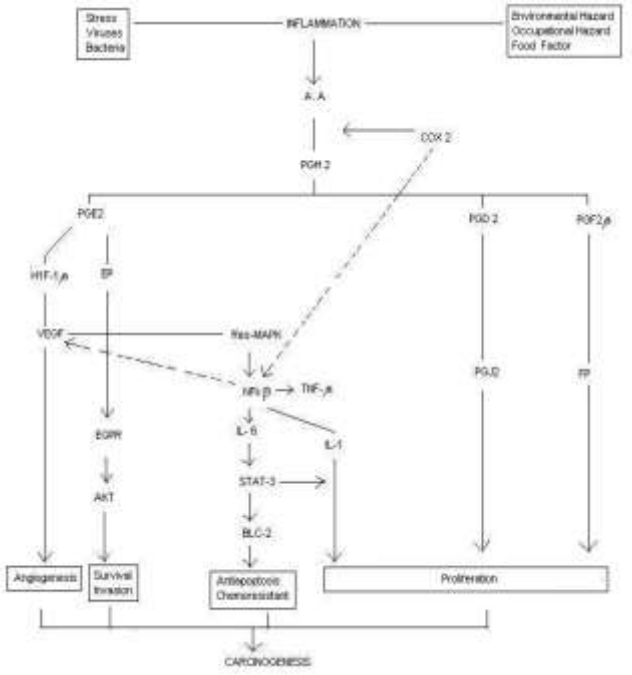

Fig.1:Complex Interaction of different inflammatory mediators showing central role of COX-2 in carcinogenesis.

CYCLOOXYGENASE ENZYME FAMILY

Cyclooxygenases (or Prostaglandin H synthases), commonly referred to as COXs, are a family of myloperoxidases located at the luminal side of the endoplasmic reticulum and nuclear membrane, which catalyze the rate limiting steps of prostaglandins biosynthesis from arachidonic acid. These enzymes act by two coupled reactions. The first one is the conversion of arachidonic acid release from the plasma membrane by phospholipase A2 to prostaglandins G2 by the cyclooxygenase activity. The second reaction is mediated by the peroxidase activity and leads to the conversion of prostaglandin G2 to prostaglandin H2. Then, different synthases convert prostaglandins H2 to prostaglandins D2, F2α, E2, I2 and thromboxane A2[19,20] as shown in fig 2.

Membrane phospholipid

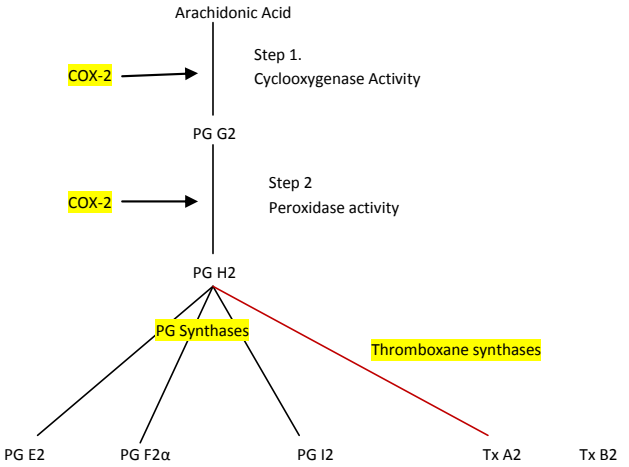

Fig 2: Diagram showing Arachidonic acid metabolism

ISOFORMS OF CYCLOOXYGENASE

At present, three COX isoenzymes are known: COX-1, COX-2, and COX-3. COX-3 is a splice variant of COX-1, which retains intron one and has a frame shift mutation; thus prefer the name COX-Ib or COX-1 variant (COX-1v). Currently the role of COX-3 is not known. Some pieces of evidences suggest a possible role in pain sensitivity, based on the studies focused on the mechanism of action of acetaminophin (paracetamol), recently evoked as a selective inhibitor of COX-3 [21]

Cycolooxygenase 1(**COX -1)**

Cycolooxygenase 1 was first purified from bovine vesicular glands in 1976. Cox 1 is on chromosome 9 (9q 32-9q33.3), contains 11 exons and spread across 40 kb; its mRNA is approximately 2.8 kb. Its molecular weight is about 75-80 kDa[22]. Cox 1 is constitutively expressed in many tissue including kidney, lung, stomach, GIT of animals and humans. It plays a role in tissue homeostasis by modulating several cellular processes ranging from cell proliferation to angiogenesis or platelet aggregation due to thromboxane production[23].

Cycolooxygenase 2(**COX-2)**

In 1989, Simmons et al[60] and in 1991 Kujubu et al[61] identified another isoform, COX-2 which shares significant sequence homology and catalytic activity with COX-1. However its expression pattern is markedly different. It is an inducible isoform, which is regulated by growth factors and different cytokines therefore over expressed during inflammation. The gene encoding COX-2 is located on chromosome 1 (1q25.2-25.3), contains 10 exons and encompasses 7.5 kb with a 4.5 kb transcript [21].

GENETIC EVIDENCE THAT COX-2 CONTRIBUTES TO CANCER

Several human and animal genetic studies have proved a direct role of COX-2 overexpression in a number of tumors, such as colorectal, breast, pancreas, lung as well as hematological malignancies like lymphomas. The first genetic evidence for a link between COX -2 and cancer comes from a $APC^{\Delta 716}$ mouse model(a model for human familial adenomatous polyposis) in which COX-2 gene was knocked out which results in the reduction of number and size of intestinal polyp[24,25]. In another study, transgenic mice was bred which over expressed the human COX-2 gene specifically in mammary glands. Multiparous females had a high frequency of focal mammary gland hyperplasia, dysplasia, and transformation into metastatic tumors[26]. In a study, forced expression of COX-2 under the control of keratin-5 promoter showed spontaneous inflammation associated urothelial cells hyperplasia and urothelial carcinomas of the urinary bladder in mice [27].

MECHANISMS BY WHICH COX-2 CONTRIBUTES TO CANCER

Cox-2 affects many processes that are important in carcinogenesis. These include xenobiotic metabolism, angiogenesis, apoptosis, anoikis, inflammation and immunosuppression[28].

Xenobiotic metabolism:

Over expression of COX-2 along with its products, prostaglandins especially PGE_2 catalyzes the conversion of procarcinogen eg., aromatic amines in cigarette, dyes and chemicals to carcinogen in organs with low P450 activity like urinary bladder. COX-2 induces the isomerization of prostaglandins endoperoxide to the mutagen melondialdehide which form adducts along the exons of the tumor suppressor genes like P53 and p16, resulting in tumor initiation and promotion[29]. Another effect is the allelic loss of chromosome 9. The 9p region contains the p16/ARF locus, and studies have confirmed that loss of these regions results in development of urothelial carcinomas[30]. In addition to catalyzing the synthesis of mutagens, COX-2 can be induced by procarcinogens. For example, benzo[a]pyrene, a polycyclic aromatic hydrocarbon in tobacco smoke and chargrilled foods, can stimulate transcription of COX-2[31]. In turn COX-2 catalyses the conversion of benzo[a]pyrene-7,8-diol to benzo[a]pyrene-7,8-diol-9,10-epoxide, which binds to DNA causing mutations[28].

Angiogenesis:

All solid malignancies are dependent on angiogenesis to grow progressively and metastasize efficiently. Over expression of COX-2 in inflammation mediated tumorigenesis leads to the production of several angiogenic inducers like αFGF, bFGF, VEGF, HGF, IL-8, TGF-β, TXA2 through prostaglandins. Through these angiogenesis mediators and their receptors on endothelial cells, COX-2 increases vascular permeability and induced endothelial cell proliferation and migration. Furthermore, over expression of COX-2 leads to the production of matrix metalloproteinase (MMPs), which have been implicated in ECM invasion[32,33,34]. Over expresion of COX-2 is also associated with increased expression of CD 44, the cell surface receptor of hyaluronate, which promote tumor cell invasion[35,40].

COX-2 mediated resistance to apoptosis:

Apoptosis is important for the development and maintnance of tissue homeostasis of multicellular organisms. Overexpression of COX-2 is associated with enhanced expression of Bcl-2 protein and decreased expression of both transforming growth factor-β2 receptors (TGFβ2) and E- cadherin. TGFβ2 receptors transduce signals that are important for inhibiting the growth of epithelial cells and E-cadherin is involved in cell – cell adhesion. Each of these changes could enhance the tumorigenic potential of epithelial cells. Upregulation of COX-2 prolonged the survival of abnormal cells, which favors the accumulations of sequential genetic changes and increases the risk of tumorigenesis[23].

Anoikis:

Anoikis is a form of apoptosis mediated by the loss of cell anchorage. This pathway plays a fundamental role during development and maintenance of tissue homeostasis by killing damaged cells or detached cells in order to maintain tissue architecture. It has been shown that anoikis is prevented in cancer cells, thus favoring tumor progression with metastasis[36]. It is also observed that possibly prostaglandins produced by COX-2 favor cell survival. A study by Joseph et al[37] showed that PGE_2 inhibit anoikis in IEC-18 cells(rat intestinal ileum cells). This effect was suggested to be due to cAMP signaling because PGE_2 receptors are coupled to adenylate cyclase, which converts AMP to cAMP. Over expression of COX-2 inhibits anoikis via activation of PI3K/Akt pathway, as the case of a human bladder cancer cell line expressing COX-2[38,39].

COX-2 and Immunosuppression:

Inflammation is associated with increased synthesis of prostaglandins through cytokines mediated induction

of COX-2, resulting in tumorigenesis. The growth of tumor is typically associated with immune suppression. Colony stimulating factors released by tumor cells activate monocytes and macrophages to synthesize prostaglandins E_2 (PGE_2), which inhibits the production of immune regulatory lymphokines, T- cell and B-cell proliferation, and the cytotoxic activity of natural killer cells. PGE_2 also inhibits the production of tumor necrosis factor α while inducing the production of IL 10, which has immunosuppresive effects [28].

COX-2 IN HUMAN NORMAL TISSUES AND OTHER CANCERS

EXPRESSION IN NORMAL TISSUES

Although immunohistochemical studies have shown that COX-2 does not express in normal human tissues, but there are other studies showing the opposite picture. For example, in a study, it is given that seminal vesicles have the high levels of constitutive expression of COX-2. PGE_2 and its 19-hydroxy metabolites are the major components of primate semen[41]. COX-2 is also constitutively expressed in human renal glomeruli and small blood vessels where it involves in sodium regulation and kidney perfusion in stress, but not in renal blood flow[42,43]. Likewise, both constitutive and inducible forms of COX-2 is present in both neuronal and non-neuronal cells in the cortex, hippocampus, hypothalamus and spinal cord, where COX-2 is involved in body temperature control and pain sensation[44]. COX-2 is also expressed in ovarian follicles upon gonadotropin stimulation, in uterine epithelial cells and surrounding stromal cells at the site of blastocyst attachment during implantation and decidualization[45].

COX-2 IN COLORECTAL CARCINOMA

Colorectal cancer is a leading cause of cancer death, with worldwide one million new cases each year and as many as half a million cancer deaths annually[46]. Cox-2 over expression in colorectal carcinoma was first reported by Eberhart et al in 1994, followed by two other groups in the next year[47,48,49]. In all these studies, COX-1 expression was found to be weak, universal and unchanged in both normal and cancerous colon, while COX-2 expression was only seen in tumors[34]. The first ever evidence linking COX-2 to carcinogenesis emerged from genetic studies on CRC. For example deletion of COX-2 gene results in decreased tumor formation in both the small intestine and colon of Apc^{min} mice (a mouse model of CRC) as well as in $APC^{\Delta 716}$ mice, another APC mutant model. Furthermore, transgenic mice with COX-2 expression driven by the keratin -5 promotors did not develop skin cancer spontaneously, but were more sensitive carcinogen induced tumor formation[24,50]. Although the role of COX-2 in CRC is still unclear but there are evidences that COX-2 dependent mechanisms appear to influence the progression from normality to neoplasia and also the replication of neoplastic cells. In this scenario, decreased apoptosis of epithelial cells secondary to COX-2 over expression appears to play a crucial role in the genesis of CRC. The gate keeping mutation required for the development of CRC is in the APC gene, which code for a pro-apoptotic protein. Modulation of apoptosis by APC involves other proteins including β-catenin and E-cadherin and the Wnt signaling pathway[51,52,53]. Besides, COX-2 potentiate the neovascularization and thus support in solid tumor growth as well as in metastasis. High micro vessel density is also found in cases of CRC[54,55].

COX-2 AND URINARY BLADDER CANCER

The natural history of urothelial carcinoma of urinary bladder is not well understood, but increased amount of COX-2 is found in both premalignant and malignant urinary bladder tissues. In a study, forced expression of COX-2 under the control of keratin-5 promoter showed spontaneous inflammation associated urothelial cells hyperplasia and urothelial carcinomas of the urinary bladder in mice[27]. The mechanism of COX-2 in bladder tumorigenesis may depends on the activation of oncogenes. Activation of K-ras gene is associated with an elevated expression of COX-2 and the K-ras gene is frequently activated in bladder tumor. Moreover, COX-2 may activates many carcinogens causing mutations in p53-a tumor suppressor gene[56,57]. Besides, COX-2 is involved in tumor invasiveness and metastasis by increasing cancer cells adhesion to extracellular matrix and decreases the level of cell adhesion molecules se.g, E-cadherin[31].

COX-2 AND BREAST CANCER

COX-2 expression was found both in *in situ* and invasive tumor as well as in poorly differentiated tumors. The mechanism by which COX-2 cause breast tumor are: inhibition of apoptosis by excess formation of PGE_2, which not only leads to increased expression of antiapoptotic protein BCL-2 and decreased expression of pro-apoptotic protein BAX and to weakening of nitric oxide signals, but also enhanced angiogenesis by forming increased VEGF, endothelin-1and PDGF; increased invasiveness by over expression of CD4; increased cell growth via estrogen receptors activation; producing mutagens by metabolism of arachidonic acid[24].

COX-2 AND GASTRIC CANCER

Although precise role of COX-2 in gastric cancer is unclear, there are evidences of missense mutations in p53

tumor suppressor gene associated with COX-2 over expression[58]. Besides, H.pylori associated upregulation of COX-2 enhances expression of VEGF which results in tumor associated micro vascular invasion. But over expression of VEGF contributes to gastric cancer invasion is still unclear. H.pylori activates NF-κB in gastric cells which in turn mediates COX-2 expression which is associated with cell proliferation[59].

COX-2 AND OTHER CANCERS

Almost all tumors express COX-2 secondary to inflammation, tumors initiators and promoters leading to decreased apoptosis, increased angiogenesis, enhanced invasion and metastasis as well as mutations in tumor suppressor genes, most important is p53.

Conclusion

Overall this review provides evidence for a strong link between COX-2 and cancer. Inflammation and cancer are both interlinked processes with lot of influence and control of several driving forces. The global burden of cancer has aroused wide concern internationally and the diagnosis and treatment for cancer are in a state of development and unfolding. It has been suggested that chemotherapy represents important modality for patients with cancers. However the results are not satisfactory due to low response rate and severe side effects. Interference with the inflammatory microenvironment has been confirmed to support antitumor activities. It is expected that rectifying the inflammatory microenvironment by using anti COX 2 drugs, potentially enhance chemotherapy of carcinomas. Since many years, we have ample knowledge about the different mechanisms by which cancer and inflammation intersect, and the time is right to translate much of the basic knowledge gained thus far and use it to add new researches to the different kinds of cancer therapies. Therefore, COX 2 is an excellent target molecule by which the detection and treatment of cancers might be possible at early stage.

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