— Review —

Pathogenic Role of Cyclooxygenase-2 in Cancer

Anantha Krishna Chaitanya Divvela,[a] Siva Reddy Challa,*,[b] and Israiel Kumar Tagaram[c]

[a]*Department of Pharmaceutical Chemistry, Shri Vishnu College of Pharmacy, Bhimavaram, PIN 534202 Andhra Pradesh, India,* [b]*Department of Pharmacology & Toxicology, Hindu College of Pharmacy, Guntur, PIN 522002 Andhra Pradesh, India and* [c]*Department of Pharmacology, Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India*

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Tremendous progress in pathogenic role of cyclooxygenase-2 (COX-2) in diverse cancers triggering the cancer research in the direction of COX-2 inhibitors. Several experimental studies reported overexpression of COX-2 in cancer cells. Mechanisms mediating the pathobiology of COX-2 in cancer are still unclear and needs to be clarified. However, recent studies have shown that the levels of COX-2 isoenzymes are elevated in certain cancers like colo-rectal carcinoma, squamous cell carcinoma of head and neck and certain cancers of lung and breast. Our review article aims to summarize the role of COX-2 in various types of cancer and mechanisms emerged from recent research and their interaction with other cytokines. It seems mechanisms mediating COX-2 and its role in each cancer may be different. In general, possible signaling from the lipids (prostaglandins) can inhibit apoptosis and increase proliferation, motility, and metastatic potential. Furthermore, under certain conditions COX-2 can contribute to angiogenesis. Even, COX-2 is found in lung cancer cells that are responsible for suppressing patients' immune systems and therefore contributing to the growth of lung cancer. COX-2 inhibitors are already in clinical trials for the prevention of colorectal, oral, skin, esophageal and non-small-cell lung cancers and for the treatment of cervical, prostate, and metastatic breast cancers. Heightened role of COX-2 in cancer prompts the pharmaceutical research to design new and safer COX-2 inhibitors to minimize the cardiovascular side effects and improves the treatment of cancer.

Key words —— prostaglandin, cyclooxygenase, tumorigenesis, carcinoma

INTRODUCTION

Prostaglandins are 20 carbon cyclopentano-fatty acid derivatives that are produced in mammalian tissue from polyunsaturated fatty acid precursors and act as autocrines or paracrines. Von Euler and Gold-blatt independently described the presence of an acidic, lipid soluble, vasodepressor and smooth muscle stimulating factor in human seminal fluid. It was believed that prostate gland was the source of this substance; hence it was called as 'prostaglandin.' Prostaglandins (PGs), in general, have a cyclopentanone ring with substituents and two side chains. By the action of cyclooxygenase (COX) isoenzymes on unsaturated fatty acid precursors these prostaglandins are formed. There are two forms COX-1 and COX-2. COX-1 is believed to be involved in the 'house keeping' functions of the body while COX-2 is expressed only under special conditions like inflammation, but recent evidences show that COX-2 also helps in the certain organs like kidneys and the brain. Most of the tissues synthesize prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2) from the respective fatty acid precursors, but their fate is different in these tissues and depends on the presence of other enzymes that convert PGH to other PGs. The biological effects of the PGs are diverse in the body, which can be explained by the presence of distinct receptors for different types of PGs that mediate their action. These receptors are named after the natural PGs for which they have greatest apparent affinity. These are divided in to five main type's viz. DP (PGD_2), FP ($PGF_{2\alpha}$), EP (PGE_2), IP (PGI_2), TP [thromboxane A_2 (TXA_2)]. The names of PGs in parentheses are the PGs that have the greatest apparent affinity for

*To whom correspondence should be addressed: Department of Pharmacology & Toxicology, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India, Tel.: +91-863-2210024; Fax: +91-863-2217783; E-mail: sivareddypharma@gmail.com

Table 1. Summary of Few Experimental Evidences of Role of COX-2 in Cancer

Year	Experimental evidence of role of COX-2 in cancer	References
1997	Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential	10
1999	Role of COX-2 was demonstrated in intestinal cancer	7
1999	Relationship between cyclooxygenase-2 expression and colo-rectal cancer was proven	11
1999	Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines	12
2000	Inhibition of COX-2 restores anti-tumor reactivity by altering the balance of IL-10 and IL-12 synthesis	13
2000	The contributions of cyclooxygenase-2 to tumor angiogenesis	14
2000	Host cyclooxygenase-2 modulates carcinoma growth	15
2000	High cyclooxygenase-2 expression in stage IB cervical cancer with lymph node metastasis or parametrial invasion	16
2001	Overexpression of cyclooxygenase-2 is sufficient to induce tumor genesis in transgenic mice	17
2002	Cyclooxygenase-2 expression in endometrial cancer	18
2003	Cyclooxygenase-2 expression in canine mammary tumors	19
2003	Expression of cyclooxygenase-1 and -2 associated with expression of VEGF in primary cervical cancer and at metastatic lymph nodes	20
2004	Expression of COX-2 protein in radio resistant laryngeal cancer	21
2004	Expression of COX-2 is increased with age in papillary thyroid cancer	22
2005	Cyclooxygenase-2 expression is associated with histologic tumor type in canine mammary carcinoma	23
2005	*Helicobacter pylori* promote gastric cancer cells invasion through a NF-κB and COX-2-mediated pathway	24
2009	Cyclooxygenase-2 expression in canine intracranial meningiomas	25

the receptors. EP receptors are further subdivided into four types viz. EP_1 (smooth muscle contraction), EP_2 (smooth muscle relaxation), EP_3 and EP_4. The COX enzymes catalyze two important steps in the PG biosynthesis. First step is the synthesis of cyclic endo-peroxide prostaglandins (PGG_2) from respective precursors by oxygenation and cyclisation catalysed by the endo-peroxide synthase component of the COX isoenzymes. The second step is the catalysed by the peroxidase component of COX isoenzymes, in which PGs of G class are reduced to form PGs of H class that are later acted upon by respective enzymes to form PG of different class. The active sites responsible for the catalysis of the two steps are different with in an isoenzyme and the active sites catalyzing the same reaction are different for the different isoenzymes.[1, 2)]

COX-2 is an enzyme that belongs to the PG G/H synthase family. It consists of 604 amino acids and has a molecular weight of 68996Da. COX-2 possesses two catalytic activities and respective active sites: COX that converts arachidonic acid to a prostaglandin endoperoxide, PGG_2, peroxidase (POX) that reduces PGG_2 to PGH_2. COX-2 functions as homodimer although each subunit has both a POX and a COX active site. Each subunit binds one heme B (iron-protoporphyrin IX) group. Wide expression in alimentary system (esophagus, pharynx), male reproductive system (prostate, seminal vesicles, ejaculatory duct), female reproductive system (cervix, uterus), hematopoietic system (bone marrow, monocytes). Enzyme that functions both as a deoxygenase and as a POX. COX-2 catalyzes the transformation of arachidonic acid (AA) to PGH_2, which is the rate-limiting step in the formation of PGs and TXA_2. COX-2 is a potent mediator of inflammation and is implicated in prostanoid signaling in activity dependent plasticity. It is an inducible enzyme that plays an important role in several pathophysiological processes, including inflammation, angiogenesis, and tumorigenesis. Five heterozygous mutations (1 missense/nonsense, 1 splicing, 3 regulatory) have been identified. Two were associated with diabetes mellitus type 2, one with bladder cancer risk, one with increased risk of colorectal cancer and one with decreased risk of colorectal cancer. It is also localized in the intracellular, cytoplasm, microsome, and microsomal membrane.[3)]

This inducible form of the enzyme, COX-2, has been implicated in cancer progression. Gately *et al.* demonstrated the contribution of COX-2 to tumor angiogenesis (shown in Table 1).[14)] COX-2 inhibitors suppress angiogenesis and tumour growth *in-vivo* (as shown in Table 2).[39)] Several mechanisms have been proposed. Side effects of the cy-

Table 2. Summary of Few Experimental and Clinical Evidences of Role of COX-2 Inhibitors in Cancer

Year	Experiemental evidence of beneficial role of COX-2 inhibitors in cancer	References
1999	Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth *in vivo*	39
2000	Celecoxib prevents tumor growth in vivo without toxicity to normal gut: lack of correlation between in vitro and in vivo models	91
2000	The effect of Celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis	92
2001	Reduced risk of Colorectal cancer among long-term users of aspirin and non-aspirin Non-steroidal anti-inflammatory drugs	93
2002	COX-2 inhibition with Celecoxib enhances anti-tumor efficacy and reduces diarrhoea side effect of CPT-11.	94
2002	Cyclo-oxygenase-2 inhibition by Celecoxib reduces proliferation and induces apoptosis in angiogenic endothelial cells in vivo	95
2002	Beneficial effect of Celecoxib on capacitabine-induced hand-and-foot syndrome and antitumor activity	96
Year	**Clinical trials of COX-2 inhibitors in cancer therapy**	**References**
2006	Celecoxib for the prevention of colorectal adenomatous polyps	97
2006	Celecoxib for the prevention of sporadic colorectal adenomas	98
2006	Reduction in the risk of human breast cancer by COX-2 inhibitors	99

clooxygenase reaction may be the problem. Many xenobiotic compounds, such as aflatoxin, are oxidized by the enzyme, forming potent mutagens. This may be particularly important in the colon, where cells are exposed to many strange molecules in the diet or the PGs themselves may be the problem. They degrade into mutagenic compounds such as malondialdehyde, which can form harmful adducts with DNA. An over abundance of PGs may also send an improper cellular signal, stimulating cell growth inappropriately or reducing the cleansing effect of apoptosis.[4] The present review provides the summary of distinct pathogenic role of COX-2 and PGs in each type of cancer though it has pathogenic similarities in all kinds of carcinogenesis.

ROLE OF PGs AND COX IN VARIOUS TYPES OF CANCER

Recent studies have shown that the levels of COX-2 isoenzymes are elevated in certain cancers like colorectal carcinoma, squamous cell carcinoma of head and neck and certain cancers of lung and breast.[5] The possible factors responsible for increased expression of COX-2 are shown in Fig. 1. In human and animal models, COX-2 levels were higher in the intestinal-type gastric adenocarcinoma and in pre-carcinogenic lesions like familial adenomatous polyposis that lead to colorectal carcinoma as compared with the normal intestinal cells. There was an upregulation of COX-2 gene expression of

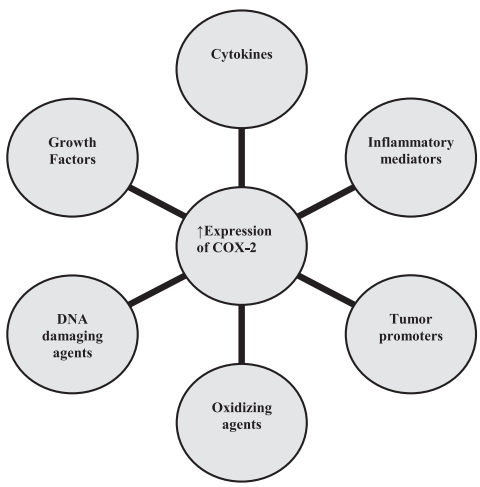

Fig. 1. Factors Responsible for Increased Expression of COX-2

human colorectal adeno carcinoma (shown in Table 3).[26] Similarly in certain squamous cell carcinoma of head and neck, levels of COX-2, PGs like $PGF_{2\alpha}$, PGE_2 and their metabolites were found to be higher than the normal tissue. Mice, in which the tumor suppressor gene APC had been knocked out, developed adenomatous polyps. The same mice, when bred with COX-2 knock out mice showed substantial decrease in polyp production.[6,7] These and other similar observations led to the hypothesis that COX-2 and certain PGs might play a crucial role in carcinogenesis. In certain experiments, PGs were found to stimulate DNA synthesis in a dose dependent manner in quiescent NIH-3T3 cells.[8] PGs and their metabolites have been shown to regulate cellular processes like mitosis, cell proliferation, and cell adhesion. Epidemiological stud-

Table 3. Summary of Few Clinical Evidences of Role of COX-2 in Cancer

Year	Clinical evidence of role of COX-2 in cancer	Reference
1994	Upregulation of cyclooxygenase-2 gene expression in human colorectal adenomas and adenocarcinomas	26
1995	Expression of cyclooxygenase-1 and -2 in human colorectal cancer	27
1997	Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential	10
2003	Role of COX-2 in carcinogenesis of colorectal cancer	28
2004	Expression of COX-2 protein in radioresistant laryngeal cancer	21

ies have suggested a decreased incidence of cancer of the esophagus, stomach, colon, and rectal in people who use a non-steroidal antiinflammatoly drug (NSAID)'s regularly, all though a delay of about a decade is seen to realize the out come. The cancers do recur and re-grow when treatment is curtailed.[9)]

COX-2 IN COLORECTAL CANCER

Colorectal cancer (CRC) remains a leading cause of cancer death, with worldwide one million new cases each year and as many as half a million cancer deaths annually.[34)] COX-2 expression is increased in the majority of colorectal tumors[26)] and this induction is associated with advanced tumor stage and correlates with poor clinical outcomes.[11)] Previous studies clearly demonstrated the COX-2 expression in colon cancer (as shown in Table 1).[10, 11)] COX-2 is undetectable in normal intestine,[35)] and its expression is significantly increased up to 85% of colorectal adenocarcinomas.[27)] In addition, COX-2 expression in human colon cancer cells increases metastatic potential (shown in Table 3).[10)] Tumor growth is dependent on angiogenesis,[36)] and several studies have indicated that higher micro vessel density (MVD) in colorectal carcinoma is associated with poor patient prognosis.[37, 38)] Recent evidence suggests that COX-2 contributes to neovascularization and may support vasculature-dependent solid tumor growth and metastasis in animal experiments[39)] and in *in vitro* studies.[10)] The first evidence linking COX-2 to carcinogenesis emerged from studies on CRC. Several subsequent reports confirmed that elevated COX-2 expression was found in approximately 50% of adenomas and 85% of adenocarcinomas. Similarly, COX-2 is induced in large intestinal epithelium in active human inflammatory bowel disease (IBD) and in inflamed tissues of interleukin (IL)-10 deficient mice (a mouse model of IBD). Direct molecular evidence that COX-2 plays a key role in colorectal carcinogenesis was obtained from studies in animal models. Genetic studies demonstrate that 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. Moreover, transgenic mice with COX-2 expression driven by the keratin-5 promoter did not develop skin cancer spontaneously, but were much more sensitive to carcinogen-induced tumor formation.[40)] In experimental studies, celecoxib prevents tumor growth *in-vivo* (shown in Table 2).[91)] Clinically, there was reduced risk of CRC among long term users of aspirin & non-aspirin NSAIDs (shown in Table 2).[93)] In support, two clinical trials reported that COX-2 inhibitors are effective for prevention of adeno colorectal carcinoma (as shown in Table 2).[97, 98)]

The role of COX-2 in carcinogenesis of CRC and its relationship with tumor biological characteristics and patients' prognosis still remain unclear. A study was designed to investigate the role of COX-2 expression in carcinogenesis of CRC and its relationship with tumor biological characteristics and patients' prognosis. COX-2 expression was detected with tissue microarray (TMA) and immunohistochemistry (IHC) procedure. The association between COX-2 expression and clinicopathological features and its influence on patients' prognosis were studied.[28)] In addition, COX-2 regulates cell cycle transition via EP_4 receptor and altered p21WAF1/CIP1 expression. Epidermal growth factor receptor (EGFR) pathways appear important. Specific targeting of the EP_4 receptor or downstream targets may offer a safer alternative to COX-2 inhibition in the chemoprevention of CRC.[33)] In brief, the association of COX-2 and colon cancer development was illustrated in Fig. 2.[100)]

PGs IN COLON CANCER

Pro-inflammatory PGE_2 plays a predominant role in promoting colorectal tumor growth. PGE_2 is

Table 4. Summary of Few Experimental Evidences of Role of Prostaglandin E_2 in Cancer

Year	Experiemental evidence of role of PG E-2 in cancer	Reference
2001	Acceleration of intestinal polyposis through prostaglandin receptor EP2 in *Apc-716* knockout mice	29
2001	Prostaglandin E2 increases growth and motility of colorectal carcinoma cells	30
2002	Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy	31
2007	Prostaglandin E2 Induces the Expression of IL 1{alpha} in colon cancer cells	32
2009	Proneoplastic effects of PGE2 mediated by EP4 receptor in colorectal cancer	33

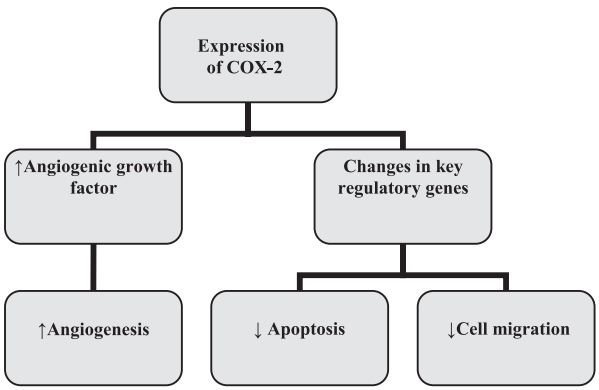

Fig. 2. Expression of COX-2 and Its Association to in Colorectal Cancer Development

the most abundant PG found in human CRC tissues. 15-PGDH is highly expressed in normal colon mucosa but is ubiquitously lost in most human CRCs. A recent study showed that PGE_2 treatment dramatically increased both small and large intestinal adenoma burden in $Apc^{Min/+}$ mice and significantly enhanced colon carcinogen azoxymethane (AOM)-induced colon tumor incidence and multiplicity. Furthermore, elevated endogenous PGE_2 via loss of 15-PGDH promoted colon tumor growth in $Apc^{Min/+}$ and AOM mouse models. The central role of PGE_2 in colorectal tumor genesis has been further confirmed by evaluating mice with homozygous deletion of PGE_2 receptors. TXA_2 has been shown to promote tumor growth and tumor-associated angiogenesis. Disruption of TP receptor doesn't affect colon tumor formation in AOM-treated mice. The role of PGD_2 in colon cancer is not defined. PGD_2 and/or its metabolites may have tumor inhibitory effects. Recent study shows that disruption of the gene for hematopoietic PGD synthase in $Apc^{Min/+}$ mice accelerates intestinal tumor growth, while $Apc^{Min/+}$ mice with transgenic human hematopoietic PGD synthase exhibit fewer intestinal adenomas than controls. These results suggest that PGD_2 serves as tumor suppressor in colorectal cancer. However, the evidence from DP receptor studies in CRC mouse models doesn't support the hypothesis that PGD_2 has tumor inhibitory effects. For example, disruption of the DP receptor didn't affect colon tumor formation in AOM-treated mice. Furthermore, there are no clear studies showing that $PGF_{2\alpha}$ participates in IBD. Similarly, the observation that $PGF_{2\alpha}$ failed to induce cell proliferation in CRC cell lines and deletion of FP receptor didn't affect colon tumor formation in AOM-treated mice suggests that $PGF_{2\alpha}$ is not involved in CRC progression. One study showed that the IP receptor is not involved in colon tumor formation in AOM-treated mice, little is known about the role of PGI_2 and IP in CRC. Since PGI_2 can activate peroxisome proliferator activated receptor-δ (PPARδ) in CRC cells and activation of PPARδ accelerates intestinal tumor growth in $Apc^{Min/+}$ mice, it is conceivable that PGI_2 may participate in the colon tumor progression through PPARδ. Further work is necessary to explore the role of PGI_2 and IP in colon carcinogenesis.[40)]

Recent studies provide strong evidence that PGE_2 is a key mediator for proneoplastic actions of COX-2. PGE_2 promotes proliferation of human colorectal carcinoma cells. DNA synthesis is increased by PGE_2 treatment in several colon cancer cell lines (as shown in Table 4).[31)] PGE_2 stimulates the growth of human colorectal cancer cells when grown in extracellular matrix.[41)] In addition, PGE_2 promotes colon cancer cell migration and increases their metastatic potential (as shown in Table 4).[30)] Further evidence demonstrates that PGE_2 promotes intestinal neoplasia through enhancing tumor angiogenesis. Knockout of the EP_2 receptor or inhibition of COX-2 enzyme results in a reduction of neoangiogenesis in *APC*716 mouse tumors (as shown in Table 4).[29)]

INTERACTIONS OF IL-1, COX-2, PGE_2 IN CANCER

The IL-1 cytokine family consists of three members, IL-1α, IL-1β, and IL-1R antagonist. IL-1 is a crucial regulator of the innate immune system

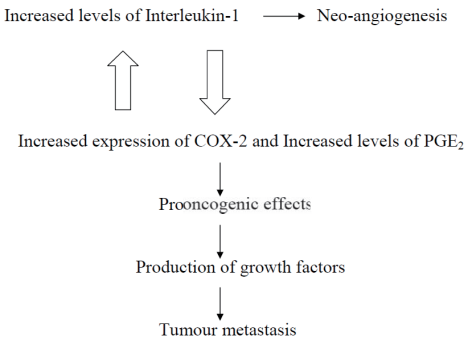

Fig. 3. Interactions between IL-1 and PGE_2

and inflammatory responses.[42,43] A variety of cell types, including lymphocytes, monocytes, fibroblasts, endothelial cells, and epithelial cells, can produce IL-1. The proinflammatory activities of IL-1 result largely from stimulating the expression of genes encoding inflammatory mediators. It is well-documented that IL-1 increases the expression of COX-2 and the production of PGE_2 (as shown in Fig. 3).[44,45] Accumulative evidence suggests that IL-1 plays critical roles in the development of malignant lesions. The most compelling evidence was generated in IL-1 knockout (KO) mouse models. Voronov *et al.*[46] have demonstrated the critical roles of IL-1 in tumor invasiveness and angiogenesis. Mice solely deficient in IL-1α or IL-1β exhibit dramatically impaired tumor development and blood vessel growth. B16 melanoma cells do not metastasize to the lung of IL-1α KO mice; however, wild-type mice die from lung metastasis by day 20 after inoculation of B16 cells. DA/3 mammary cancer cells fail to grow tumors in the foot pad of IL-1α KO mice, whereas progressive tumor growth is observed in wild type mice. In support of these findings, the expression of IL-1 is significantly increased in a variety of malignant lesions and particularly in metastatic human tumor specimens.[47,48]

PGE_2 has been shown to exert pro-oncogenic effects in colorectal neoplasia through producing autocrine or paracrine growth factors. An investigation was conducted to demonstrate the PGE_2 induced the expression of IL-1α in colon cancer cells, which plays critical roles in tumor metastasis and neoangiogenesis in a variety of cancers. PGE_2 increased the levels of IL-1α, mRNA and protein, suggesting a positive feedback loop between the IL-1 pathway and PGE_2 signaling. Mechanistically, PGE_2 induced the expression of IL-1α at both transcriptional and posttranscriptional levels. PGE_2 stimulated the transcriptional activity of the IL-1α promoter and significantly stabilized IL-1α mRNA. Moreover, we show that IL-1α enhanced colorectal neoplasia, stimulating cell migration and neoangiogenesis. Knockdown of the expression of IL-1α by small-interfering RNA resulted in a reduction of vascular endothelial growth factor secretion in colon cancer cells. Finally PGE_2 induces the expression of proinflammatory cytokine IL-1α, which may potentially enhance the proneoplastic actions of the COX-2/PGE_2 signaling pathway.[32]

COX-2 AND BONE CANCER

Bone cancer pain is one of the most difficult of all persistent pain states to fully control. Common sequels of malignant tumors in bone include severe pain, skeletal fractures, bone marrow suppression, hypercalcemia and an overall reduced quality of life.[49] Several tumor types including sarcomas and breast, prostate, and lung carcinomas grow in or preferentially metastasize to the skeleton where they proliferate and induce significant bone remodeling, bone destruction and cancer pain. Many of these tumors, as well as sensory and spinal cord neurons, express the isoenzyme COX-2 which is involved in the synthesis of PGs. To begin to define the role prostaglandins play in driving bone cancer, bone remodeling and bone cancer pain, we used an *in vivo* model where murine osteolytic 2472 sarcoma cells, which were stably transfected with green fluorescent protein (GFP), were injected and confined to the intramedullary space of the femur of male C3HHeJ mice. Following tumor implantation, mice develop ongoing and movement-evoked bone cancer pain-related behaviors, extensive tumor-induced bone resorption, infiltration of the marrow space by tumor cells, and stereotypic alterations in the spinal cord reflective of a persistent pain state. Thus, following injection of tumor cells, bone destruction is first evident at day 6 and pain-related behaviors are maximal at day 14.[50]

COX-2 AND BREAST CANCER

In breast cancer, COX-2 act as follows: inhibition of apoptosis by induction of PGE_2, which leads to increased expression of antiapoptotic protein BCL-2 and decreased expression of proapoptotic protein BAX and to weakening of nitric oxide (NO) signals; enhanced angiogenesis due to increased PGE_2 level, followed by increased VEGF,

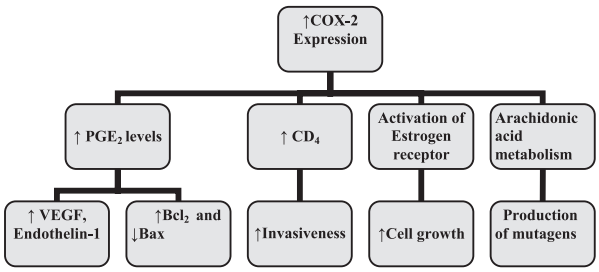

Fig. 4. Expression of COX-2 and Its Association with Formation of Breast Cancer

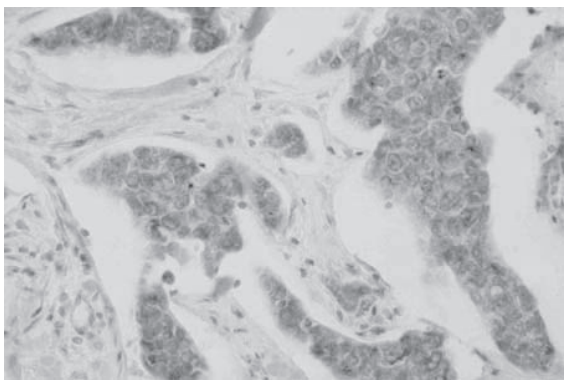

Fig. 5. Moderate to Hight Cytoplasmic Expression of COX-2 in Invasive Ductal Breast Carcinoma

endothelin-1 and PDGF production; increased invasiveness via over expression of CD_4; increased cell growth via estrogen receptor activation; producing mutagens by metabolism of AA (as shown in Fig. 4).

Howe and Dannenberg found that elevated levels of COX-2 protein correlate with tumor size, high proliferation rate, axillary node metastases, histology, human epidermal growth factor receptor 2 (HER-2) gene amplify cation and decreased disease-free survival in breast cancer.[51)] Association of high COX-2 expression with reduced disease-free survival and also with disease-related survival was also found in estrogen receptor (ER) negative breast cancers.[52)] Expression of COX-2 in cancer cells varied from 5% to 100% in reviewed papers with an average score of 40%.[51–53)] COX-2 expression was found both in invasive and in *in situ* breast cancer[54)] and, in poorly differentiated carcinomas, the intensity of expression was significantly higher. Adjacent, non-neoplasmic tissues were negative for COX-2 staining.[55)] As in these studies, results on TMAs of 32 cases of invasive ductal carcinomas show diff use strong cytoplasmatic, granular expression of COX-2 in all studied tumors (as shown in Fig. 5).[56)] The role of COX-2 gene polymorphism in breast cancer development is also a matter of current discussion. COX-2 is also a target for therapy by selective (celecoxib) or non-selective non-steroid anti-inflammatory drugs (aspirin, indomethacin) in several diseases and their protective contribution against the development of various tumor types has been shown in animal mouse and rodent models.[53)] Studies on rodent breast cancer have shown a significant decrease in the incidence, multiplicity and volume of tumor after selective NSAIDs treatment.[51, 53)]

COX-2 AND LARYNGEAL CANCER

The precise mechanistic role for COX-2 in laryngeal cancer continues to be evaluated; however, it's over expression is believed to play an important role.[57)] In support of this, Oshim *et al.* have demonstrated that 'knocking out' the COX-2 gene leads to a marked reduction in intestinal polyps, in a mouse model of familial adenomatous polyposis.[58)] COX-2 over expression has also been implicated in tumor response to radiotherapy. Tsujii and Dubois demonstrated that cell lines which over express COX-2 were resistant to apoptosis, an important pathway of cell death induced by ionizing radiation.[59)] Pyo *et al.* demonstrated that the COX-2 inhibitor, NS-398, enhances the effect of radiotherapy *in vitro* and *in vivo* on human cells that over express COX-2.[60)] The radiation-enhancing effects of NS-398 did not occur in cells deficient in COX-2 expression and concluded that COX-2 over expression was essential for the effects of NS-398. On the basis of these observations an investigation was done to determine the possible relationship between COX-2 protein expression and treatment failure in laryngeal cancer treated with radiotherapy.

Using immune histochemical techniques the expression of COX-2 protein in 122 pre-treatment laryngeal biopsies were examined. All tumors were treated with single modality radiotherapy (curative intent). The group comprised of 61 radio resistant and 61 radiosensitive tumors matched for T stage, laryngeal subsite, gender and smoking history. COX-2 expression was detected in 41 of 61 (67%) biopsy samples from patients with radio resistant tumors and 25 of 61 (41%) radiosensitive tumors. Over expression was significantly associated with radio resistant tumors ($p = 0.004$). COX-2 has 67% accuracy in predicting radiotherapy failure. COX-2 may have prognostic value in predicting response to radiotherapy. COX-2 inhibitors such as NS-398 have been shown to enhance the effects of

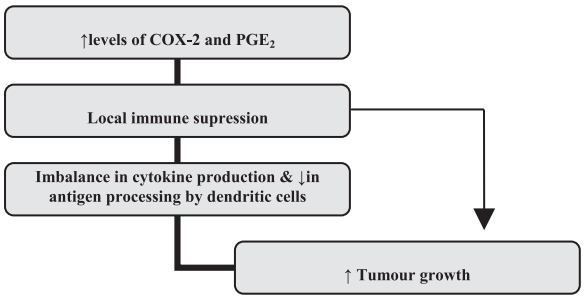

Fig. 6. Role of COX-2 and PGE_2 Induction of Lung Cencer

radiotherapy (as shown in Table 1).[21)]

COX-2 AND LUNG CANCER

Ermert *et al.* report in this issue the expression patterns of COX-1, COX-2, PGE_2 synthase, PGD_2 synthase, prostaglandin I-synthatase (PGI-S), and TXA_2 synthase in a variety of lung tumors. The role of COX-2 and PGs in lung cancer is now attracting a considerable amount of attention from cancer biologists and the public. COX-2 can be induced by cytokines, growth factors, oncogenes, and tumor promoters.[61)] Elevated levels of COX-2[62)] and PGE_2[63)] are found in non-small cell lung carcinoma (NSCLC), resulting in local immune suppression, a condition that favors tumor growth. This idea is supported by the findings of Huang *et al.*[62)] and Stolina *et al.*[64)] that showed that human lung cancer cell-derived PGE_2 caused an imbalance in cytokine production and a reduction in antigen processing by dendritic cells (as shown in Fig. 6). Moreover, in a murine Lewis lung carcinoma model, treatment with anti-PGE_2 monoclonal antibody retarded the growth rate of tumors, leading to prolonged survival.[13)]

COX-2 AND OVARIAN CANCER

Endometrial cancer (endometrial adenocarcinoma) is the most common type of female genital cancer worldwide. Most endometrial neoplasias are diagnosed while they are still restricted to the uterus, although endometrial cancer may spread along the uterine cavity to the cervix, penetrate the uterine wall, or spread through the fallopian tubes. Once disseminated, it is as lethal as ovarian cancer.[65)]

In vivo and *in vitro* studies have shown that COX-2 expression is implicated in vascular endothelial growth factor (VEGF) expression in many cancers in terms of angiogenesis.[18, 66)] In a limited number of studies about the relationship between VEGF and COX in gynecological malignancy, VEGF has been found to correlate with COX-2 in endometrial cancer (as shown in Table 1)[18)] and with COX-1 in ovarian cancer.[20)] However, VEGF expression in endometrial cancer along with that of COX-1 and COX-2 has not been adequately analyzed.

COX-2 AND GASTRIC CANCER

Gastric cancer is one of the most common malignant gastrointestinal tumors; the incidence and mortality of gastric cancer show an upward tendency. The unlimited growth of the tumor cell and the metastasis are the important trait of the tumor, are the main cause of cancer related death induced by the failure of treatment of gastric cancer.

VEGF also plays a vital role in tumor-associated micro vascular invasion. In human gastric cancers, VEGF has been found to be over-expressed, and in a recent study, VEGF expression has been reported to be upregulated by *Helicobacter pylori* (*H. pylori*) through a COX-2 dependent mechanism. Whether VEGF contributes to gastric cancer invasion induced by *H. pylori* infection remains unknown. Several recent studies suggested that COX-2 might be an important factor in carcinogenesis, and COX-2 inhibitors were shown to possess anticancer effects. These properties were mediated through the inhibition of PGs production by COX-2, leading to decreases in angiogenic factors, and changes in matrix metallo proteinase (MMP) activity. In human gastric cancer cells, nuclear factor (NF)-κB mediated COX-2 expression is associated with cell proliferation. Furthermore, *H. pylori* activate NF-κB expression in gastric cancer cells.[24)]

COX-2, PGE_2 AND PROSTATE CANCER

Dietary fatty acid intake is associated with the risk of development and progression of prostate, colon and breast cancer.[67)] *In vitro* and *in vivo* studies suggest that the availability of polyunsaturated fatty acids contributes to increased cancer cell growth, and that inhibitors of the eicosanoids synthesis pathway inhibit cell proliferation.[68)] In human prostate tissues, PGE_2 is the only signifi-

cant eicosanoid produced.[69] PGE_2 induces a variety of cell responses depending on the tissue type and the receptors involved, such as immune regulation, smooth muscle and regulation of water readsorption in kidney. Previous studies with osteoblasts and prostate cancer cells have demonstrated that PGE_2 stimulates cell growth.[70] Studies from this laboratory indicate that AA stimulates growth of a prostate cell line, PC-3 (manuscript submitted). Because of the role of PGE_2 in prostate cancer cell growth, we hypothesized that AA had the stimulatory effect on the prostate cancer cells through the activity of its metabolite PGE_2, and aimed to understand the signal transduction events following AA administration which lead to the growth of the cells. After being synthesized and exported out of the cells, PGE_2 exerts its functions by interacting with the PGE_2 receptors (EPs), which G-protein coupled receptors which activates Gs protein. Four subtypes of EP receptors have been identified and characterized. These receptors are coupled to G proteins, and activate or inhibit second messenger systems inside the cell. EP_1 causes influx of Ca^{2+} and activation of protein kinase C (PKC); receptors EP_2 and EP_4 activate the adenylate cyclase which increases cellular cyclic adenosin 5′-monophosphate (AMP) level and activates protein kinase A (PKA); EP_3 signals primarily through an inhibitory G protein to decrease intracellular cyclic AMP levels.[71] Despite the important association of AA and PGE_2 with prostate cancer, the signals mediating the biological functions of these molecules in prostate cancer cells are not fully understood. The signalling pathways mediating AA- or PGE_2-induced cell growth or expression of growth-related proto-oncogenes have been investigated in a number of studies with varying conclusions. In bone cells, a PKA-mediated mechanism has been suggested.[72] A study of smooth muscle cells demonstrated a role for PKC as a mediator of AA-induced *c-fos* expression 102. In the Swiss 3T3 fibroblast cells, however, there are conflicting data on whether PKC or PKA is involved.[73, 74]

COX-2 AND PAPILLARY THYROID CANCER

Papillary thyroid cancer (PTC) is a unique cancer for two reasons; first, its prognosis depends largely on age, and secondly, lymph node metastases (LNM) do not necessarily indicate poor survival. Many authors have reported that lymph node metastases are associated with an increased rate of loco regional recurrence of disease but not with survival.[75] COX-2 expression is higher in the tumors from older PTC patients and that this could explain the more aggressive behavior of PTC in the older age group (as shown in Table 1).[22] COX-2 is involved in the formation of prostanoids from AA and, in normal tissues, it is induced in response to a wide range of cellular signals. Higher COX-2 expression correlates with a poor clinical outcome in various cancers,[11] and selective inhibition of COX-2 suppresses tumor growth.[12] Moreover, COX-2 expression is associated with LNM.[16] Recently, a novel correlation of COX-2 with VEGF-C was reported in human lung adenocarcinoma, and COX-2 was found to up-regulate VEGF-C expression.[76] Later, correlation of VEGF-C with COX-2 was also demonstrated in head and neck squamous cell carcinoma and in oesophageal squamous cell carci noma.[77] VEGF-C, originally found in the human prostatic adenocarcinoma cell line,[78] stimulates lymphatic proliferation and is expressed in cancers metastasizing to lymph nodes.[79] Papillary thyroid cancer also expresses VEGF-C. Some of these studies have also demonstrated correlations between lymph node metastasis and high VEGF-C levels, but no correlations with other clinical parameters.[80, 81]

COX-2 AND MAMMARY CARCINOMA

Cellular conditions, such as hypoxia, cytokines (IL-6), oncogenes (ras and scr), and VEGF, lead to increased COX-2 expression.[82] At the cellular level, higher activity of COX-2 helps promote tumor invasiveness that is mediated through increased activity of matrix metalloproteinases 2 and 9.[83] COX-2 promotes expression of the anti-apoptotic protein bcl-2, thereby inhibiting cellular senescence.[14] The study by Dore *et al.*[19] indicated an association between degrees of COX-2 expression when comparing mammary adenomas with mammary carcinomas. However, to date and the author's knowledge, there have been no studies evaluating the association between levels of COX-2 expression and tumor histologic subtype of canine mammary carcinoma. Histologic subtype is a known prognostic variable in canine mammary carcinoma, with solid and anaplastic carcinomas carrying a worse prognosis, compared with that for tubular or papillary adenocarcinomas.[84]

The role of COX-2 in canine mammary neoplasia remains to be more clearly elucidated by Heller *et al.* It clearly reported whether a direct association between levels of COX-2 expression and tumor histologic subtype exists in canine mammary carcinoma. Immuno-histochemical analysis was performed using a polyclonal anti PG G/H synthase 2 IgG COX-2 antibody. Sections from the kidneys of young dogs, which stain positive for COX-2 in the macula densa, served as positive controls. Positive staining tumors were given a COX-2 staining distribution and intensity score according to previously established scales. The product of the COX-2 staining distribution and intensity scores was calculated to create COX-2 staining distribution and intensity scores was calculated to create COX-2 expression was detected. Anaplastic carcinomas had a significantly higher COX-2 staining distribution intensity and index, compared with those for adenocarcinomas.[23] However, clinical trials indicated the reduction in the risk of human breast cancer by COX-2 inhibitors (as shown in Table 2).[99]

COX-2 AND OTHER CANCERS

Expression of COX-2 is induced by many physiological and stress signals including growth factors, cytokines and other mediators of inflammation, tumor promoters, oxidizing agents and DNA damaging agents.[26] Many of the signals that activate COX-2 also induce tumor suppressor p53. The p53 is a transcription factor that induces antiproliferative responses such as cell cycle arrest, DNA repair, or apoptosis in response to DNA damage.[85] The p53 pathway is disturbed in practically all main types of human cancer.[86]

PGE_2 AND LEUKEMIA

PGs E_1, E_2, and $F_{2\alpha}$ (PGE_1, PGE_2, and $PGF_{2\alpha}$) were shown to inhibit the growth of mouse leukemia lymphoblasts L5178Y in culture. The effects of PGE_1 and PGE_2 were greater than that of $PGF_{2\alpha}$. The ability of the cells to form colonies in soft agar was significantly inhibited by PGE_1 and PGE_2 at concentrations as low as 1.8 μg/ml. For $PGF_{2\alpha}$, however, a concentration as high as 56 μg/ml was required to show inhibitory effect, but at 1.8 μg/ml it was found to be stimulatory.[87]

PGE_2 IN CANCER ASSOCIATED IMMUNODEFICIENCY

Tumor-induced immune suppression is a fundamental problem in cancer biology and immunotherapy. COX metabolites act as tumor promoters when overproduced[15, 17] and recent studies have demonstrated that the COX metabolite PGE_2 exhibits potent immunosuppressive effects, orchestrating an imbalance between type 1 and type 2 cytokines.[64, 88] Importantly, PGE_2 has been shown to be a key modulator of dendritic cell (DC) function, altering cytokine production as well as the I-A^d class II cell surface marker.[89] The contribution of the PGE_2 EP_2 receptor to cancer-associated immune deficiency using $EP_2^{-/-}$ mice. $EP_2^{-/-}$ mice exhibited significantly attenuated tumor growth and longer survival times when challenged with MC26 or Lewis lung carcinoma cell lines as compared with their wild-type littermates. While no differences in T cell function were observed, PGE_2 suppressed differentiation of DC from wild-type bone marrow progenitors, whereas EP_2-null cells were refractory to this effect. *In vivo*, DCs, CD_4 and CD_8 T cells were significantly more abundant in draining lymph nodes of tumor-bearing EP_2 mice than in tumor-bearing wild-type mice, and a significant antitumor cytotoxic T-lymphocyte response could be observed only in the $EP_2^{-/-}$ animals. This confirms the important role for the EP_2 receptor in PGE_2-induced inhibition of DC differentiation and function and the diminished antitumor cellular immune responses *in vivo.*[90]

PG INHIBITORS AS ANTICANCER AGENTS

Cyclo-pentanone PGs, mainly of A_1 and J_2 types have shown to have anti-viral and anti-cancer activity. Certain cyclopentenone prostaglandins with cross-conjugated dienone systems like PGJ_2 and PGA_1 act on certain intra-cellular targets within the nucleus and inhibit tumors. These PGs arrest the cell growth in tumors at the G1/S interphase of the cell cycle.

CONCLUSION

Involvement of COX-2 in various cancers with distinct mechanisms has been proved based on the

experimental evidences of recent studies. It is very clear that COX-2 is one of the important targets for the treatment of cancer. Though, COX-2 inhibitors like Celecoxib have shown significant efficacy in cancer patients, its use is limited by cardiovascular side effects. Designing safer COX-2 inhibitors is a future challenge to improve the pharmacotherapy of cancer.

CLINICAL TRIALS AND FUTURE PERSPECTIVES

COX-2 is overexpressed in human colorectal adenomas and tumours, but not in normal colorectal tissue, indicating that COX-2 inhibitors might prevent colorectal cancer. The results of two large trials that investigated the use of the COX-2 inhibitor celecoxib for the prevention of colorectal adenomas were reported recently. These trials were both stopped in late 2004 following reports that celecoxib and other COX-2 inhibitors could increase the risk of cardiovascular events. Two clinical trials [Adenoma Prevention with Celecoxib (APC) and Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP)] revealed that celecoxib was significantly more effective than placebo for preventing adenomas over 3 years of treatment. In the APC trial, the cumulative incidence of adenomas was 60.7% for patients who received placebo, and 43.2% and 37.5% for patients who received 200 mg or 400 mg of celecoxib twice a day, respectively. In the PreSAP trial, the cumulative incidence of adenomas was 49.3% in the placebo group and 33.6% in the celecoxib group. Both trials were too small to assess whether celecoxib decreased CRC rates, but reduction in adenoma is considered an excellent surrogate endpoint for CRC. However, both trials also found that celecoxib was associated with an increased risk of cardiovascular events compared with placebo. A hypothetical risk-benefit analysis in an accompanying editorial suggested that celecoxib could have an advantage over no treatment for preventing CRC. However, because cardiovascular events are much more common than CRC, the increase in cardiovascular events with celecoxib clearly outweighed any possible decrease in CRC. So, although celecoxib can prevent colorectal adenomas, it cannot be recommended for this indication owing to an increased risk of cardiovascular events. Considering the significance of COX-2 inhibitors in both the preventive and therapeutic aspects, it is important to design the safer COX-2 inhibitors to improve the treatment aspects of cancer.

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