Pathogenic Role of Cyclooxygenase-2 in Cancer

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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 polysaturated 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 G2 (PGG2) and prostaglandin H2 (PGH2) 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 (PGD2), FP (PGF2α), EP (PGE2), IP (PGI2), TP [thromboxane A2 (TXA2)]. The names of PGs in parentheses are the PGs that have the greatest apparent affinity for...
### Table 1. Summary of Few Experimental Evidences of Role of COX-2 in Cancer

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<thead>
<tr>
<th>Year</th>
<th>Experimental evidence of role of COX-2 in cancer</th>
<th>References</th>
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<tbody>
<tr>
<td>1997</td>
<td>Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential</td>
<td>10</td>
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<td>1999</td>
<td>Role of COX-2 was demonstrated in intestinal cancer</td>
<td>7</td>
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<td>1999</td>
<td>Relationship between cyclooxygenase-2 expression and colo-rectal cancer was proven</td>
<td>11</td>
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<tr>
<td>1999</td>
<td>Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines</td>
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<td>2000</td>
<td>Inhibition of COX-2 restores anti-tumor reactivity by altering the balance of IL-10 and IL-12 synthesis</td>
<td>13</td>
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<tr>
<td>2000</td>
<td>The contributions of cyclooxygenase-2 to tumor angiogenesis</td>
<td>14</td>
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<tr>
<td>2000</td>
<td>Host cyclooxygenase-2 modulates carcinoma growth</td>
<td>15</td>
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<tr>
<td>2000</td>
<td>High cyclooxygenase-2 expression in stage IB cervical cancer with lymph node metastasis or parametral invasion</td>
<td>16</td>
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<tr>
<td>2001</td>
<td>Overexpression of cyclooxygenase-2 is sufficient to induce tumor genesis in transgenic mice</td>
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<td>2002</td>
<td>Cyclooxygenase-2 expression in endometrial cancer</td>
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<td>2003</td>
<td>Cyclooxygenase-2 expression in canine mammary tumors</td>
<td>19</td>
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<tr>
<td>2003</td>
<td>Expression of cyclooxygenase-1 and -2 associated with expression of VEGF in primary cervical cancer and at metastatic lymph nodes</td>
<td>20</td>
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<td>2004</td>
<td>Expression of COX-2 protein in radio resistant laryngeal cancer</td>
<td>21</td>
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<tr>
<td>2004</td>
<td>Expression of COX-2 is increased with age in papillary thyroid cancer</td>
<td>22</td>
</tr>
<tr>
<td>2005</td>
<td>Cyclooxygenase-2 expression is associated with histologic tumor type in canine mammary carcinoma</td>
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<tr>
<td>2005</td>
<td><em>Helicobacter pylori</em> promote gastric cancer cells invasion through a NF-κB and COX-2-mediated pathway</td>
<td>24</td>
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<tr>
<td>2009</td>
<td>Cyclooxygenase-2 expression in canine intracranial meningiomas</td>
<td>25</td>
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**the receptors. EP receptors are further subdivided into four types viz. EP<sub>1</sub> (smooth muscle contraction), EP<sub>2</sub> (smooth muscle relaxation), EP<sub>3</sub> and EP<sub>4</sub>. The COX enzymes catalyze two important steps in the PG biosynthesis. First step is the synthesis of cyclic endo-peroxide prostaglandins (PGG<sub>2</sub>) 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.<sup>1,2</sup>**

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<sub>2</sub>, peroxidase (POX) that reduces PGG<sub>2</sub> to PGH<sub>2</sub>. 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<sub>2</sub>, which is the rate-limiting step in the formation of PGs and TXA<sub>2</sub>. 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.<sup>3</sup>

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).<sup>14</sup> COX-2 inhibitors suppress angiogenesis and tumour growth *in-vivo* (as shown in Table 2).<sup>39</sup> 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

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

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. 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 human colorectal adeno carcinoma (shown in Table 3). 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 polypt production. 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. 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

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

ies have suggested a decreased incidence of cancer of the esophagus, stomach, colon, and rectal in people who use a non-steroidal antiinflammatoty 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 tumors26) 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 experiments39) 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<sup>Min</sup> mice (a mouse model of CRC) as well as in Apc<sup>∆716</sup> 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 adenocarcinoma (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<sub>4</sub> receptor and altered p21WAF1/CIP1 expression. Epidermal growth factor receptor (EGFR) pathways appear important. Specific targeting of the EP<sub>4</sub> 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<sub>2</sub> plays a predominant role in promoting colorectal tumor growth. PGE<sub>2</sub> is
Table 4. Summary of Few Experimental Evidences of Role of Prostaglandin E2 in Cancer

<table>
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<th>Year</th>
<th>Experimental evidence of role of PG E-2 in cancer</th>
<th>Reference</th>
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<tbody>
<tr>
<td>2001</td>
<td>Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc-716 knockout mice</td>
<td>29</td>
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<tr>
<td>2001</td>
<td>Prostaglandin E2 increases growth and motility of colorectal carcinoma cells</td>
<td>30</td>
</tr>
<tr>
<td>2002</td>
<td>Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy</td>
<td>31</td>
</tr>
<tr>
<td>2007</td>
<td>Prostaglandin E2 Induces the Expression of IL 1{alpha} in colon cancer cells</td>
<td>32</td>
</tr>
<tr>
<td>2009</td>
<td>Proneoplastic effects of PGE2 mediated by EP4 receptor in colorectal cancer</td>
<td>33</td>
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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 peroxisomal proliferator activated receptor-$\delta$ (PPAR$\delta$) in CRC cells and activation of PPAR$\delta$ accelerates intestinal tumor growth in Apc$^{Min/+}$ mice, it is conceivable that PGI$_2$ may participate in the colon tumor progression through PPAR$\delta$. 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). PGE$_2$ stimulates the growth of human colorectal cancer cells when grown in extracellular matrix. In addition, PGE$_2$ promotes colon cancer cell migration and increases their metastatic potential (as shown in Table 4). 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 APC716 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-1$\alpha$, IL-1$\beta$, and IL-1R antagonist. IL-1 is a crucial regulator of the innate immune system...
Interactions between IL-1 and PGE2

and inflammatory responses. 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 PGE2 (as shown in Fig. 3). 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) mice models. Voronov et al. 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.

PGE2 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 PGE2 induced the expression of IL-1α in colon cancer cells, which plays critical roles in tumor metastasis and neoangiogenesis in a variety of cancers. PGE2 increased the levels of IL-1α, mRNA and protein, suggesting a positive feedback loop between the IL-1 pathway and PGE2 signaling. Mechanistically, PGE2 induced the expression of IL-1α at both transcriptional and posttranscriptional levels. PGE2 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 PGE2 induces the expression of proinflammatory cytokine IL-1α, which may potentially enhance the proneoplastic actions of the COX-2/PGE2 signaling pathway.

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. 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.

COX-2 AND BREAST CANCER

In breast cancer, COX-2 act as follows: inhibition of apoptosis by induction of PGE2, which leads to increased expression of antipapoptotic protein BCL-2 and decreased expression of proapoptotic protein BAX and to weakening of nitric oxide (NO) signals; enhanced angiogenesis due to increased PGE2 level, followed by increased VEGF,
endothelin-1 and PDGF production; increased invasiveness via over expression of CD4; 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 amplification and decreased disease-free survival in breast cancer. 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. Expression of COX-2 in cancer cells varied from 5% to 100% in reviewed papers with an average score of 40%. COX-2 expression was found both in invasive and in in situ breast cancer and, in poorly differentiated carcinomas, the intensity of expression was significantly higher. Adjacent, non-neoplastic tissues were negative for COX-2 staining. 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). 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. Studies on rodent breast cancer have shown a significant decrease in the incidence, multiplicity and volume of tumor after selective NSAIDs treatment.

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

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

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. 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. 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. 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. 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 and 25 of 61 (41%) radiosensitive tumors. 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...
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-synthetase (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$_2$ 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.

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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.$^{65}$ 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-
PGE₂ 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 re-absorption in kidney. Previous studies with osteoblasts and prostate cancer cells have demonstrated that PGE₂ stimulates cell growth. Studies from this laboratory indicate that AA stimulates growth of a prostate cell line, PC-3 (manuscript submitted). Because of the role of PGE₂ in prostate cancer cell growth, we hypothesized that AA had the stimulatory effect on the prostate cancer cells through the activity of its metabolite PGE₂, 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, PGE₂ exerts its functions by interacting with the PGE₂ 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. EP₁ causes influx of Ca²⁺ and activation of protein kinase C (PKC); receptors EP₂ and EP₄ activate the adenylate cyclase which increases cellular cyclic adenosin 5′-monophosphate (AMP) level and activates protein kinase A (PKA); EP₃ signals primarily through an inhibitory G protein to decrease intracellular cyclic AMP levels. Despite the important association of AA and PGE₂ 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 PGE₂-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. 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.

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. 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). COX-2 is involved in the formation of prostanooids 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, and selective inhibition of COX-2 suppresses tumor growth. Moreover, COX-2 expression is associated with LNM. 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. Later, correlation of VEGF-C with COX-2 was also demonstrated in head and neck squamous cell carcinoma and in oesophageal squamous cell carcinoma. VEGF-C, originally found in the human prostatic adenocarcinoma cell line, stimulates lymphatic proliferation and is expressed in cancers metastasizing to lymph nodes. 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.

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. 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. COX-2 promotes expression of the anti-apoptotic protein bcl-2, thereby inhibiting cellular senescence. The study by Dore et al. 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.
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 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 anti-proliferative 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)

PGE₂ AND LEUKEMIA

PGs E₁, E₂, and F₂α (PGE₁, PGE₂, and PGF₂α) were shown to inhibit the growth of mouse leukemia lymphoblasts L5178Y in culture. The effects of PGE₁ and PGE₂ were greater than that of PGF₂α. The ability of the cells to form colonies in soft agar was significantly inhibited by PGE₁ and PGE₂ at concentrations as low as 1.8 µg/ml. For PGF₂α, 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)

PGE₂ IN CANCER ASSOCIATED IMMUNODEFICIENCY

Tumor-induced immune suppression is a fundamental problem in cancer biology and immunotherapy. COX metabolites act as tumor promoters when overproduced15,17) and recent studies have demonstrated that the COX metabolite PGE₂ exhibits potent immunosuppressive effects, orchestrating an imbalance between type 1 and type 2 cytokines.64,88) Importantly, PGE₂ has been shown to be a key modulator of dendritic cell (DC) function, altering cytokine production as well as the I-A<sup>d</sup> class II cell surface marker.89) The contribution of the PGE₂ EP<sub>2</sub> receptor to cancer-associated immune deficiency using EP<sub>2</sub><sup>−/−</sup> mice. EP<sub>2</sub><sup>−/−</sup> 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, PGE₂ suppressed differentiation of DC from wild-type bone marrow progenitors, whereas EP<sub>2</sub>-null cells were refractory to this effect. In vivo, DCs, CD<sub>4</sub> and CD<sub>8</sub> T cells were significantly more abundant in draining lymph nodes of tumor-bearing EP<sub>2</sub> mice than in tumor-bearing wild-type mice, and a significant antitumor cytotoxic T-lymphocyte response could be observed only in the EP<sub>2</sub><sup>−/−</sup> animals. This confirms the important role for the EP<sub>2</sub> receptor in PGE<sub>2</sub>-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<sub>1</sub> and J<sub>2</sub> types have shown to have anti-viral and anti-cancer activity. Certain cyclopentenone prostaglandins with cross-conjugated diene systems like PGJ<sub>2</sub> and PGA<sub>1</sub> 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.

**REFERENCES**


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