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Selective administration of COX-2 inhibitors play potential role in prostate cancer management- An "In silico" analysis

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Abstract
Tumorigenesis is a complex process, and understanding the mechanisms behind tumorigenesis is key to identifying effective targeted therapies. Prostaglandins are signaling lipophilic molecules derived from phospholipids that are involved in normal physiologic functions. However, overexpression of prostaglandins has been associated with tumorigenesis. Several epidemiologic studies have shown an inverse correlation between the incidence of colon cancer and the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandin synthesis. The NSAIDs target cyclooxygenases (COX), essential enzymes in prostaglandin production. Cyclooxygenase-2 (COX-2) is an inducible form of the enzyme that is usually not expressed in normal tissue. Because COX-2 is frequently overexpressed in premalignant lesions and neoplasms, specific COX-2 inhibitors have been investigated as chemoprevention and potential chemotherapeutic agents. There is now preclinical and early clinical data that suggest inhibitors of COX-2 may protect against colon, breast, lung, esophageal, and oral tumors. This paper will discuss evidence addressing the possible mechanistic contribution of COX-2 in tumorigenesis and will explore the link between COX-2 activity and carcinogenesis. The potential role of COX-2 inhibitors in the chemoprevention and treatment of various tumors will also be discussed. Clinical trials using targeted inhibitors of COX-2 will be critical in determining if COX-2 is a viable molecular target in cancer management.

Keywords: Prostaglandins, cyclooxygenase (COX), apoptosis, angiogenesis, tumorigenesis

Introduction
Prostaglandins and their derivatives are signaling lipophilic molecules that are involved in diverse homeostatic and reactive functions, including platelet aggregation, clot formation, vasodilation, and gastric cytotoxic protection, as well as renal sodium and water balance. The first step in the synthesis of prostaglandins is the hydrolysis of membrane phospholipids to arachidonic acid by phospholipase A_2.Arachidonic acid is then converted to an unstable product, prostaglandin G_2, which is rapidly converted to prostaglandin H_2 by the peroxidase activity of a cyclooxygenase (COX). Prostaglandin H_2 is converted by tissue-specific enzymes to other prostaglandins and thromboxanes. There are two isoforms of the COX enzyme encoded by two different genes. Cyclooxygenase-1 (COX-1) is a constitutive enzyme that is present in most normal tissues and mediates the synthesis of prostaglandins required for normal physiologic functions. The gene for COX-1 is on human chromosome 9 [1]. Cyclooxygenase-2 (COX-2) is an inducible form of the enzyme that is not normally detected in most tissues and is coded by a gene on human chromosome 1 [2]. Cyclooxygenase-2 is induced by cytokines, growth factors, tumor promoters, and carcinogens [3]. Cyclooxygenase-2 is also induced by several oncogenes, such as v-Src, v-Ha-*ras*, HER2/*neu* and Wnt [4-7].
Although prostaglandins are involved in many normal physiologic functions, these molecules—and the COX enzymes involved in prostaglandin production may also be involved in tumorigenesis. Indeed, there is evidence that prostaglandins may contribute to tumorigenesis by inhibiting the immune system, stimulating cell growth, enhancing angiogenesis, increasing mutagen production, enhancing cell invasion, or inhibiting apoptosis. In this article, we will discuss these mechanisms and will explore the role of COX-2 tumorigenesis. We will also review clinical trials that have investigated COX-2inhibitors as chemo protection agents and will discuss future directions in the study of COX-2 as a therapeutic target in cancer management.

Mechanisms of Prostaglandins in Carcinogenesis
Immunosuppressive Effects
Prostaglandins have a variety of immunosuppressive effects. For example, prostaglandin E_2 diminishes the cytotoxic activity of natural killer cells, inhibits T-cell and B-cell growth, and decreases the production of cytokines including tumor necrosis factor-alpha. Huang *et al.,* [8]. showed that pretreatment with a prostaglandin inhibitor prevents an increase in interleukin-10 synthesis by peripheral blood lymphocytes. Furthermore, prostaglandins may also interfere with antigen processing by dendritic cells [9]. The ability of prostaglandins to inhibit the immune system may allow tumors to grow without surveillance and may contribute to tumorigenesis.

Mitogenic Effects
Enhanced prostaglandin synthesis may also contribute to tumorigenesis by direct stimulation of cell growth. Data have shown that prostaglandin E_2 and prostaglandin F_2-alpha can be Mitogenic in BALB/c3T3fibroblasts in the presence of epidermal growth factor [10]. Furthermore, proliferation of mammary epithelial cells can be stimulated in the presence of epidermal growth factor by prostaglandin E_1 and prostaglandin

E_2 [11]. in breast tissue, prostaglandins may stimulate cell growth by stimulating the aromatase gene, CYP19, and thus enhancing estrogen synthesis [12, 13]. Interestingly, enhanced expression of CYP19 and COX has been found in human breast cancer specimens [14].

Inhibition of Apoptosis

Prolonged survival of abnormal cells favors the accumulation of genetic changes that may result in tumor formation. Therefore, inhibition of apoptosis may increase the tumorigenic potential of initiated cells. Sheng *et al.*, [15]. demonstrated that prostaglandin E_2 may inhibit apoptosis by inducing bcl-2. Recently it was shown that inhibition of COX-2 by celecoxib (Celebrex), as elective COX-2 inhibitor, resulted in a decrease of production of prostaglandin E_2 and TXB_2, and was associated with an increase in apoptosis in vivo [16].

Mechanisms of Cyclooxygenase in Carcinogenesis

Effects on Metastatic Potential

Enhanced COX-2 expression may also contribute to tumorigenesis by increasing cell invasiveness. DuBois *et al.*, [17]. Showed that COX-2overexpression in rat intestinal epithelial cells increases cell adhesion to the extracellular matrix. Recently, Stockton and Jacobson [18] demonstrated that COX-2 is required for NIH3T3 cell migration in a process that appears to be regulated by the extracellular signal-regulated kinase 1/2. Furthermore, the activity of enzymes responsible for digesting cellular basement membrane is enhanced by COX-2 overexpression in the breast cancer cell line Hs578T and colon cancer cells, which likely contributes to the increased ability of these cells to invade through a layer of Matrigel [19, 20].

FASTA

cyclooxygenase-2 [Homo sapiens]

GenBank: AAA58433.1

GenPept Identical Proteins Graphics

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>gi|181254|gb|AAA58433.1| cyclooxygenase-2 [Homo sapiens]
MLARALLLCAVLALSHTANPCCSHPCQNRGVCMSVGFDQYKCDCTRTGFYGENCSTPEFLTRIKLFLKPT
PNTVHYILTHFKGFWNVVNNIPFLRNAIMSYVLTSRSHLIDSPPTYNADYGYKSWEAFSNLSYYTRALPP
VPDDCPTPLGVKGKKQLPDSNEIVGKLLLRRKFIPDPQGSNMMFAFFAQHFTHQFFKTDHKRGPAFTNGL
GHGVDLNHIYGETLARQRKLRLFKDGKMKYQIIDGEMYPPTVKDTQAEMIYPPQVPEHLRFAVGQEVFGL
VPGLMMYATIWLREHNRVCDVLKQEHPEWGDEQLFQTSRLILIGETIKIVIEDYVQHLSGYHFKLKFDPE
LLFNKQFQYQNRIAAEFNTLYHWHPLLPDTFQIHDQKYNYQQFIYNNSILLEHGITQFVESFTRQIAGRV
AGGRNVPPAVQKVSQASIDQSRQMKYQSFNEYRKRFMLKPYESFEELTGEKEMSAELEALYGDIDAVELY
PALLVEKPRPDAIFGETMVEVGAPFSLKGLMGNVICSPAYWKPSTFGGEVGFQIINTASIQSLICNNVKG
CPFTSFSVPDPELIKTVTINASSSRSGLDDINPTVLLKERSTEL
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Fig 1: Human Cox-2 sequence

Increased Production of Mutagens

Another mechanism by which COX-2 overexpression may play a role in carcinogenesis is an increase in the production of mutagens. One such potent mutagen is malondialdehyde, which can be produced by isomerization of prostaglandin H_2. Malondialdehyde acts by forming adducts with deoxynucleotides, which cause frame-shifts and base-pair substitutions [21] In addition, Wiese *et al.*, [22] showed that the peroxidase activity of cyclooxygenases can catalyze the formation of mutagens by the oxidation of aromatic amines, heterocyclic amines, and dihydrodiol derivatives of polycyclic hydrocarbons. Thus, COX-2overexpression may lead to DNA damage that may eventually lead to carcinogenesis.

Angiogenesis

Cyclooxygenase-2 has also been implicated in enhanced angiogenesis, which plays a role in carcinogenesis. Tumor growth depends on increased blood supply via secretion of angiogenic promoters such as vascular endothelial growth factor. Cyclooxygenase-2 overexpression in colon cancer cells is correlated with increased production of vascular growth factors and formation of capillary networks [23]. This angiogenic effect of COX-2 can be blocked byNS398, a selective COX-2 inhibitor. Williams *et al.*, [24]. Demonstrated that tumor formation is markedly decreased in COX-2 knockout mice compared with wild-type mice. Furthermore, the pharmacologic inhibition of COX-2 leads to a decrease in vascular endothelial growth factor production that may contribute to a decrease in tumor formation. Masferrer *et al.*, [25]. showed that celecoxib, a selective COX-2inhibitor, blocks corneal blood vessel formation in a rat model. Thus, COX-2overexpression may increase tumor blood supply and may contribute to tumor growth.

Further Links between COX-2 and Tumorigenesis

Cyclooxygenase-2 is upregulated in multiple human premalignant and malignant conditions, including tumors of the colon, breast, stomach, lung, pancreas, cervix, prostate, bladder, liver, skin, head and neck, and esophagus [26-40]. There are multiple lines of evidence suggesting a link between

levels of COX-2 and tumorigenesis. Increased levels of COX-2 are detected in premalignant intestinal tumors in experimental animal models [41]. The knockout of the COX-2 gene led to a marked reduction in the size and number of polyps in APCD^{716} mice. In addition, APCD^{716} mice treated with a selective COX-2 inhibitor had reduced adenoma formation [42]. Further, COX-2 knockout mice develop fewer skin papillomas than control mice [43]. Taken together, these results suggest that inhibition of COX-2 could be important in the prevention of a variety of epithelial tumors.

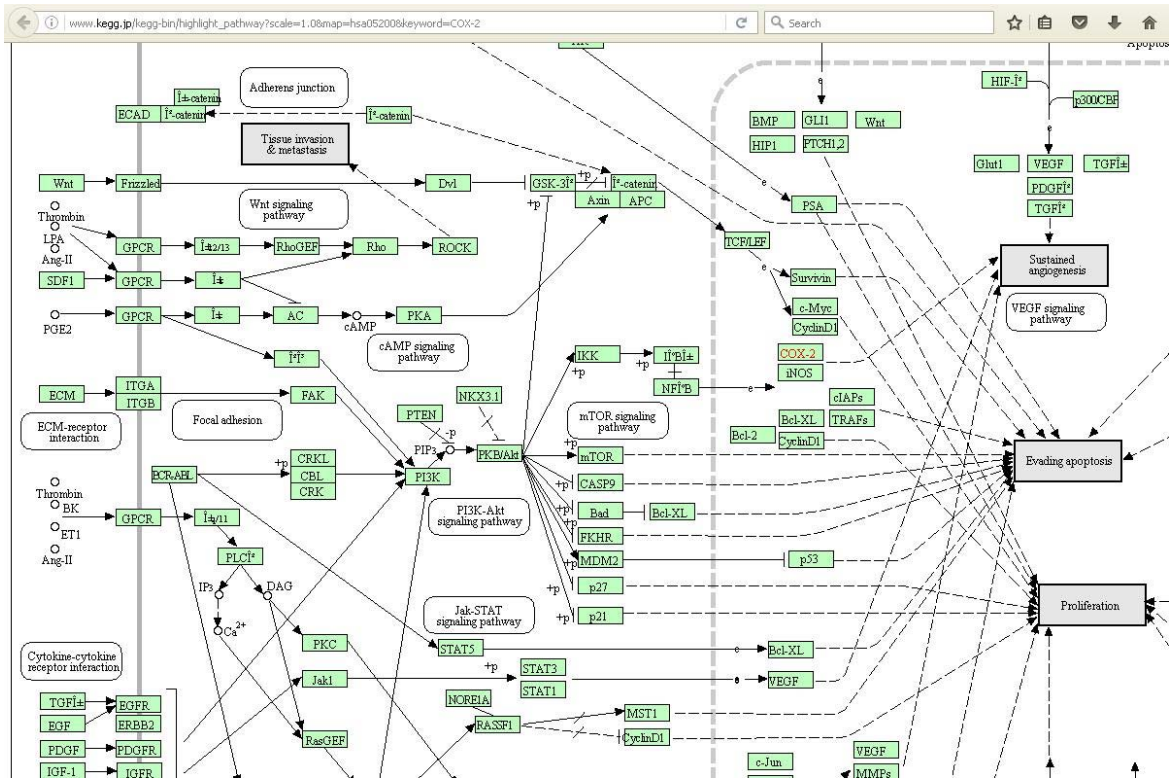

Fig 2A: Metabolism of human COX-2 Pathway

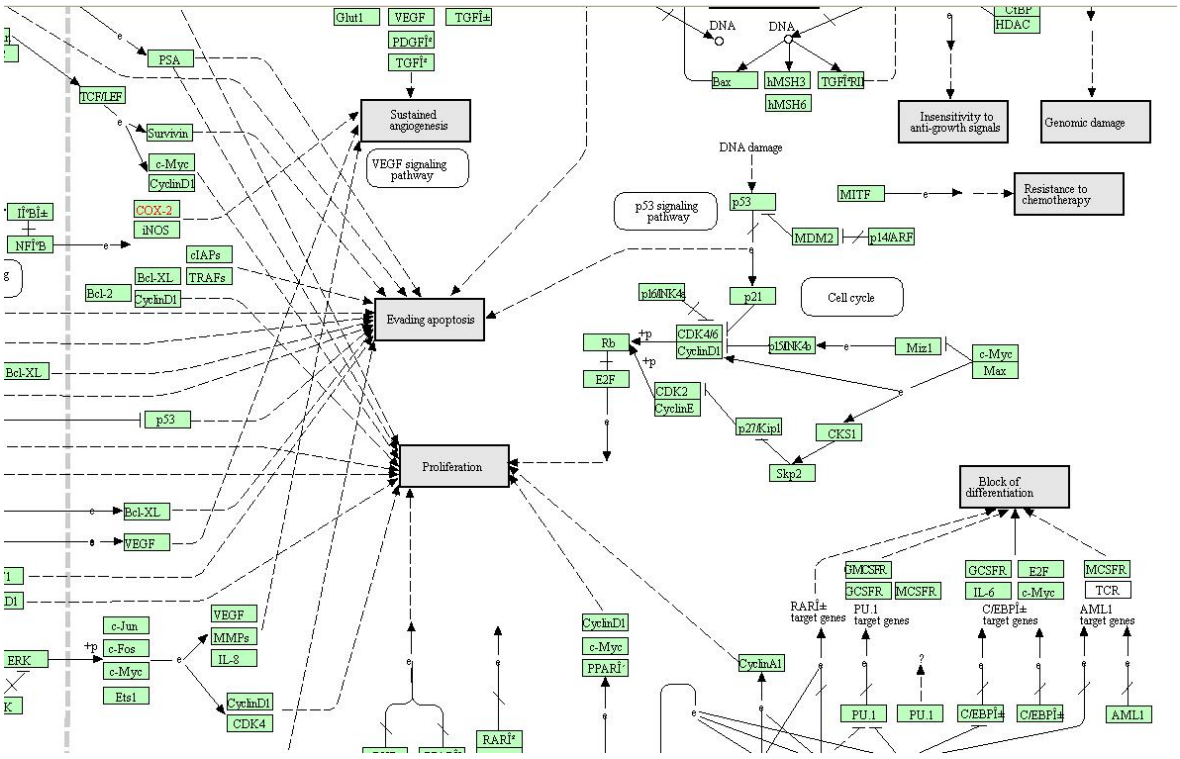

Fig 2B: Rest part of Metabolism of human COX-2 Pathway

Synthetic and naturally occurring inhibitors of COX (e.g. sulindac [Clinoril], ibuprofen, flurbiprofen [Ansaid], indomethacin [Indocin])have also been shown to protect against mammary, colon, oral, and esophageal cancer in experimental animals [44-50]. For example, studies have shown that flurbiprofen, an inhibitor of COX-1 and COX-2, can inhibit the growth of transplanted mammary tumors [51] and increase the mean survival duration in mice [52]. In addition to having therapeutic activity against established mammary tumors, flurbiprofen inhibits mammary carcinogenesis induced by a low dose of N-methyl-N-nitrosourea in rats [46]. Other data have also demonstrated that indomethacin, another inhibitor of both COX-1 and COX-2, has significant chemo protective activity in rats when administered during either the early or late stage of mammary tumorigenesis [47]. Importantly, epidemiologic studies have shown that chronic intake of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the incidence of various human cancers, including cancers of the colon, breast, lung, esophagus, stomach, and bladder [53-60]. Celecoxib has been evaluated as a possible chemo preventive agent for the inhibition of tumorigenesis. In a study to evaluate the inhibitory activity of celecoxib against azoxymethane-induced aberrant crypt foci formation in the colon of rats, celecoxib significantly suppressed the total number of aberrant crypt foci in rats compared with placebo [61]. In a second study of azoxymethane-treated rats, celecoxib reduced the incidence of colon cancer by 93% and tumor multiplicity by 97%. [62]. these animal data provide strong evidence that celecoxib has cancer activity in the prevention of cancer when tested in a defined model of tumorigenesis. Recently, Harris *et al*, [63]. Compared the chemoprevention effects of celecoxib with ibuprofen and placebo in the development and growth of7, 12-dimethyl-benz (a) anthracene (DMBA)-induced rat mammary tumors. Seven days prior to receiving a single dose of 15 mg of DMBA by intubation, rats were fed either a control diet or diets containing 1,500 ppm of celecoxib or 1,500 ppm of ibuprofen. Both celecoxib and ibuprofen significantly increased tumor latency, reduced tumor burden, and prevented tumor formation compared with placebo. However, celecoxib was more potent than ibuprofen. At 105 days after DMBA treatment, tumor incidence was 100% in control rats compared with 32% and 60% in rats fed either celecoxib (P<.001) or ibuprofen (P<.001), respectively. The control rats had an average of 3.2 tumors compared with 0.4and 1.5 tumors in rats treated with celecoxib (P<.001) and ibuprofen (P<.001), respectively. Additionally, the average tumor volume was 1.5 cm^3 in control rats vs 0.3 cm^3 and 0.6 cm^3 in rats treated with celecoxib (P<.001) or ibuprofen (P<.001), respectively. The higher potency of celecoxib suggests that this agent may have an in vivo advantage over other NSAIDs as a chemoprevention agent [63]. The DMBA-induction model was also used to evaluate celecoxib for efficacy against established tumors. Alshafie *et al.*, [64] examined the effect of celecoxib, given as a daily diet (1,500 ppm), on the growth of established DMBA-induced tumors in rats over a 6-week treatment period. Tumors in the control group continued to grow; whereas tumors in the celecoxib group markedly decreased in size. The average reduction in tumor volume relative to baseline was approximately 32% (P<.04). At the end of the 6-week treatment period, average tumor volume was 1.45 cm^3 and 0.13 cm^3 in the control and celecoxib groups, respectively. Tumor volume regression occurred in 90% of rats treated with celecoxib [64]. These results suggest that celecoxib has potent antitumor activity and chemoprevention activity in this rat mammary carcinoma model.

Clinical Trials Using COX-2 Inhibitor

Chemoprevention Trials

Familial adenomatous polyposis (FAP) is a rare disease that accounts for approximately 1% of colorectal carcinomas annually. In individuals with FAP, numerous reports have documented the chemoprevention effects of sulindac and other NSAIDs on existing adenomas. Waddell *et al.*, [65] was the first to describe near-complete regression of colorectal adenomas in four patients treated with sulindac. Subsequent studies have confirmed significant reductions in colorectal adenomas in individuals treated with sulindac [66]. Recently, celecoxib was evaluated in a randomized, double-blind, placebo-controlled trial in 77 FAP patients [67]. In this study, patients were randomized to receive 100- or 400-mg celecoxib twice daily or placebo for 6months. Patients treated with 100-mg celecoxib experienced reductions in the number of colorectal polyps and total polyp burden, although these changes did not reach statistical significance. Patients treated with 400-mg celecoxib achieved a 28% reduction in the number of colorectal polyps vs a 4.5% reduction in patients treated with placebo (P =.003) [67]. Additionally, the total polyp burden (sum of polyp diameters) was significantly lower in those patients treated with 400-mg celecoxib compared with patients in the placebo group (30.7% vs 4.9%, P =.001). Furthermore, a higher proportion of patients treated with 400-mg celecoxib experienced at least a 25% reduction in polyp number compared with patients treated with placebo (53% ersus 7%, P =.003).Celecoxib at 400 mg twice daily also improved the endoscopic appearance of both the colorectum and duodenum of patients with FAP. These results in humans are consistent with previous results using selective COX-2 inhibitors to prevent intestinal tumors in experimental animals [42, 62]. Based on the results of this trial, celecoxib was approved for the treatment of FAP. Because of the similarities in the biology of sporadic colorectal cancer and FAP, agents that are effective in FAP may also be useful for chemoprevention of sporadic colorectal adenomas. Chemoprevention of sporadic colorectal tumors with NSAIDs has been evaluated in three small, uncontrolled studies, with mixed results, [68-70]. Only one large, placebo-controlled trial has investigated NSAIDs for chemoprevention of sporadic colorectal cancer thus far. The Physicians' Health Study investigated whether aspirin was effective, but showed no significant difference in self-reported frequency of new colorectal malignancies in patients treated with aspirin (325 mg every other day for 5years) compared with placebo [71]. Presently, several ongoing clinical trials are evaluating the efficacy of selective COX-2 inhibitors for the prevention of sporadic colorectal adenomas [72]. Because the majority of colorectal cancers arise from preexisting adenomas, results from these trials are highly anticipated. Several lines of data suggest that COX-2 inhibitors may be effective in other diseases including Barrett's esophagus, oral leukoplakia, actinic keratoses, and bladder cancer. Cyclooxygenase-2 is overexpressed in Barrett's esophagus and oral leukoplakia, [39, 40] and NSAIDs have been shown to prevent esophageal and oral cancers in animal models [45, 73] Preclinical data have also

shown that COX-2 inhibitors protect against ultraviolet light-induced skin cancer formation in mice, [74] suggesting that a COX-2 inhibitor may be effective in the treatment of actinic keratoses, a premalignant skin lesion. Furthermore, there are clinical data demonstrating the benefit of the topical NSAID diclofenac (Solaraze) in the treatment of actinic keratosis [75]. Cyclooxygenase-2 is also overexpressed in bladder cancer [36]. And selective COX-2 inhibitors have been shown to protect against bladder tumorigenesis in animal models [76]. Based on these data, the National Cancer Institute has initiated several clinical trials to further investigate the use of selective COX-2inhibitors as chemoprevention agents in these diseases. The results of these ongoing clinical trials will be important in determining the role of COX-2inhibitors in the prevention of certain cancers.

Chemotherapeutic Trials

Cyclooxygenase-2 inhibitors are also actively being investigated as chemotherapeutic agents. Many preclinical studies have demonstrated thatCOX-2 inhibitors delay tumor progression but have less of an effect on tumor regression. However, COX-2 inhibitors may enhance the effects of standard anticancer therapy. Several preclinical studies have demonstrated the synergistic antitumor efficacy of a COX-2 inhibitor when given concurrently with chemotherapy or radiation. [77, 78] Recently, Subbaramaiah *et al.,* [79] have demonstrated a link between the overexpression of the HER2/*neu* oncogene and the up regulation ofCOX-2 in human breast cancer. In this study, increased expression of COX-2 is detected in nearly 100% of HER2/*neu*-overexpressing human breast cancers. In contrast, COX-2 is only expressed at very low levels in a minority of cases ofHER2/*neu*-normal breast cancer. This is the first study to demonstrate a clear link between overexpression of HER2/*neu* and up regulation of COX-2 in breast cancer. To elucidate the mechanism by which HER2/*neu* regulates COX-2expression, a cell culture model was used. Increased levels of prostaglandin E2production, COX-2 protein, and COX-2 mRNA were detected in HER2/*neu*-transformed human mammary epithelial cells (184B5/HER) compared with their non-transformed counter parts (184B5) [79]. Based on this study, a clinical trial is under way to assess the combined efficacy of celecoxib and trastuzumab (Herceptin) in patients with metastatic HER2/*neu*-overexpressing breast cancer.

Future Directions

Significant progress has been made in our understanding of COX-2and its role in tumorigenesis. As noted, COX-2 overexpression and prostaglandin synthesis may contribute to tumorigenesis via increased cellular proliferation, diminished immune surveillance, decreased apoptosis, enhanced cell invasiveness, increased mutagen production, and effects on angiogenesis [6, 8-11, 13, 14, 17-25, 80] although the relative importance of each of these effects is unknown. Clinical trials are ongoing to evaluate specific COX-2 inhibitors for cancer chemoprevention in the colorectum, esophagus, skin, prostate, and bladder. Based on the available data, clinical studies should also be considered to evaluate COX-2 inhibitors in the prevention of cancer of the lung, pancreas, cervix, liver, stomach, and breast because COX-2 is also overexpressed in tumors involving these organs [28-34, 37]. Combination therapy with established chemotherapeutic agent's andCOX-2 inhibitors also should be explored. Several preclinical reports have shown that taxanes can induced COX-2 expression [81-83]. Thus, the up regulation of COX-2may decrease the antitumor efficacy of taxanes and, perhaps, the combination of a COX-2 inhibitor with taxanes may provide additive or synergistic antineoplastic effects. Furthermore, the arthralgia/myalgia syndrome that is often noted in patients treated with taxanes may also be ameliorated with aCOX-2 inhibitor.

Conclusion

Cyclooxygenase-2 inhibitors are relatively safe in humans and are an exciting new class of drugs with potential for cancer management, either alone or in combination with standard therapies. However, the exact role that COX-2 inhibitors may play in the current armamentaria of anticancer agents remains to be fully elucidated.

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References

1. Funk CD, Funk LB, Kennedy ME. Human platelet/erythroleukemia cell prostaglandin G/H synthase: cDNA cloning, expression, and gene chromosomal assignment. FASEB J. 1991; 5:2304-2312.
2. Kosaka T, Miyata A, Ihara H. Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. Eur J Biochem 1994; 221:889-897.
3. Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta 1996; 1299:125-140,
4. Xie W, Herschman HR. v-Src induces prostaglandin synthase 2 gene expression by activation of the c-Jun N-terminal kinase and the c-Jun transcription factor. J Biol Chem. 1995; 270:27622-27628.
5. Vadlamudi R, Mandal M, Adam L. Regulation of cyclooxygenase-2 pathway by HER2 receptor. Oncogene 1999; 18:305-314.
6. Sheng H, Williams CS, Shao J. Induction of cyclooxygenase-2 by activated Ha-ras oncogene in Rat-1 fibroblasts and the role of mitogen-activated protein kinase pathway. J Biol Chem. 1998; 273:22120-22127.
7. Howe LR, Subbaramaiah K, Chung WJ. Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. Cancer Res 1999; 59:1572-1577.
8. Huang M, Sharma S, Mao JT. Non-small-cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol. 1996; 157:5512-5520.

9. Stolina M, Sharma S, Zhu L. Lung cancer cyclooxygenase-2 dependent inhibition of dendritic cell maturation and function. Pro Am Assoc Cancer Res 2000; 41:619.
10. Nolan RD, Danilowicz RM, Eling TE. Role of arachidonic acid metabolism in the mitogenic response of BALB/c 3T3 fibroblasts to epidermal growth factor. Mol Pharmacol 1988; 33:650-656.
11. Bandyopadhyay GK, Imagawa W, Wallace D. Linoleate metabolites enhance the in vitro proliferative response of mouse mammary epithelial cells to epidermal growth factor. J Biol Chem. 1987; 262:2750-2756.
12. Harris RE, Robertson FM, Abou-Issa HM. Genetic induction and up regulation of cyclooxygenase (COX) and aromatase (CYP19): An extension of the dietary fat hypothesis of breast cancer. Medical Hypotheses 1999; 52:291-292.
13. Zhao Y, Agarwal VR, Mendelson CR. Estrogen biosynthesis proximal to a breast tumor is stimulated by PG E2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 1996; 137:5739-5742.
14. Brueggemeier RW, Quinn AL, Parrett ML. Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens. Cancer Lett 1999; 140:27-35.
15. Sheng H, Shao J, Morrow JD. Modulation of apoptosis and bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 1998; 58:362-366.
16. Leahy K, Ornberg R, Wang Y. Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in epithelial cells. Cancer Res 2002; 62:625-631.
17. DuBois RN, Shao J, Tsujii M. G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. Cancer Res 1996; 56:733-737.
18. Stockton RA, Jacobson BS. Modulation of cell-substrate adhesion by arachidonic acid: Lipoxygenase regulates cell spreading and erk1/2-inducible cyclooxygenase regulates cell migration in NIH3T3 fibroblasts. Mol Biol Cell 2001; 12:1937-1956.
19. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA 1997; 94:3336-3340.
20. Takahashi Y, Kawahara F, Noguchi M. Activation of matrix metalloproteinase-2 in human breast cancer cells overexpressing cyclooxygenase-1 or -2. FEBS Lett 1999; 460:145-148.
21. Marnett LJ. Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res 1992; 52:5575-5589.
22. Wiese FW, Thompson PA, Kadlubar FF. Carcinogen substrate specificity of human COX-1 and COX-2. Carcinogenesis 2001; 22:5-10.
23. Tsujii M, Kawano S, Tsuji S. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998; 93:705-716.
24. Williams CS, Tsujii M, Reese J. Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest. 2000; 105:1589-1594.
25. Masferrer JL, Leahy KM, Koki AT. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000; 60:1306-1311.
26. Eberhart CE, Coffey RJ, Radhika A. Upregulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994; 107:1183-1188.
27. Sano H, Kawahito Y, Wilder RL. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995; 55:3785-3789.
28. Hwang D, Scollard D, Byrne J. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst. 1998; 90:455-460.
29. Soslow RA, Dannenberg AJ, Rush D. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2000; 89:2637-2645.
30. Ristimaki A, Honkanen N, Jankala H. Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 1997; 57:1276-1280.
31. Hida T, Yatabe Y, Achiwa H. Increased expression of cyclooxygenase-2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 1998; 58:3761-3764.
32. Wolff H, Saukkonen K, Anttila S. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 1998; 58:4997-5001.
33. Tucker ON, Dannenberg AJ, Yang EK. Cyclooxygenase-2 expression is upregulated in human pancreatic cancer. Cancer Res 1999; 59:987-990.
34. Kulkarni S, Rader JS, Zhang F. Cyclooxygenase-2 is overexpressed in human cervical cancer. Clin Cancer Res 2001; 7:429-434.
35. Gupta S, Srivastava M, Ahmad N. Overexpression of cyclooxygenase-2 in human prostate adenocarcinoma. Prostate 2000; 42:73-78.
36. Mohammed SI, Knapp DW, Bostwick DG. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res 1999; 59:5647-5650.
37. Koga H, Sakisaka S, Ohishi M. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: Relevance to tumor dedifferentiation. Hepatology 1999; 29:688-696.
38. Buckman SY, Gresham A, Hale P. COX-2 expression is induced by UVB exposure in human skin: Implications for the development of skin cancer. Carcinogenesis 1998; 19:723-729.
39. Chan G, Boyle JO, Yang EK. Cyclooxygenase-2 expression is upregulated in squamous cell carcinoma of the head and neck. Cancer Res 1999; 59:991-994.
40. Wilson KT, Fu S, Ramanujam KS. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. Cancer Res 1998; 58:2929-2934.
41. Williams CS, Luongo C, Radhika A. Elevated cyclooxygenase-2 levels in Min mouse adenomas. Gastroenterology 1996; 111:1134-1140.
42. Oshima M, Dinchuk JE, Kargman SL. Suppression of intestinal polyposis in APC delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996; 87:803-809.
43. Tiano H, Chulada P, Spalding J. Effects of cyclooxygenase deficiency on inflammation and papilloma development in mouse skin. Proc Am Assoc Cancer Res 1997; 38:17-27.

44. Reddy BS, Maruyama H, Kelloff G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug, during different stages of rat colon tumor development. Cancer Res 1987; 47:5340-5346.
45. Cornwall H, Odukoya O, Shklar G. Oral mucosal tumor inhibition by ibuprofen. J Oral Maxillofac Surg 1983; 41:795-800.
46. McCormick DL, Moon RC. Inhibition of mammary carcinogenesis by flurbiprofen, a nonsteroidal anti-inflammatory agent. Br J Cancer. 1983; 48:859-861.
47. McCormick DL, Madigan MJ, Moon RC. Modulation of rat mammary carcinogenesis by indomethacin. Cancer Res 1985; 45:1803-1808.
48. Boolbol SK, Dannenberg AJ, Chadburn A. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 1996; 56:2556-2560,
49. Perkins TM, Shklar G. Delay in hamster buccal pouch carcinogenesis by aspirin and indomethacin. Oral Surg Oral Med Oral Pathol 1982; 53:170-178.
50. Huang MT, Lou YR, Ma W. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 1994; 54:5841-5847.
51. Leaper DJ, French BT, Bennett A. Breast cancer and prostaglandins: A new approach to treatment. Br J Surg. 1979; 66:683-686.
52. Bennett A, Berstock DA, Carroll MA. Increased survival of cancer-bearing mice treated with inhibitors of prostaglandin synthesis alone or with chemotherapy. Br J Cancer 1982; 45:762-768.
53. Thun MJ, Namboodiri MM, Heath CW. Jr Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991; 325:1593-1596.
54. Greenberg ER, Baron JA, Freeman DH. Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. J Natl Cancer Inst 1993; 85:912-916.
55. Logan RF, Little J, Hawtin PG. Effect of aspirin and nonsteroidal anti-inflammatory drugs on colorectal adenomas: Case-control study of subjects participating in the Nottingham faecal occult blood screening programme. BMJ 1993; 307:285-289.
56. Suh O, Mettlin C, Petrelli NJ, Aspirin use, cancer, and polyps of the large bowel. Cancer 1993; 72:1171-1177.
57. Coogan PF, Rao SR, Rosenberg L. The relationship of nonsteroidal anti-inflammatory drug use to the risk of breast cancer. Prev Med 1999; 29:72-76.
58. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994; 5:138-146.
59. Castelao JE, Yuan JM, Gago-Dominguez M. Nonsteroidal anti-inflammatory drugs and bladder cancer prevention. Br J Cancer 2000; 82:1364-1369.
60. Farrow DC, Vaughan TL, Hansten PD. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998; 7:97-102.
61. Reddy BS, Rao CV, Seibert K: Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996; 56:4566-4569.
62. Kawamori T, Rao CV, Seibert K. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998; 58:409-412.
63. Harris RE, Alshafie GA, Abou-Issa H. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000; 60:2101-2103.
64. Alshafie GA, Abou-Issa HM, Seibert K. Chemotherapeutic evaluation of celecoxib, a cyclooxygenase-2 inhibitor, in a rat mammary tumor model. Oncol Rep 2000; 7:1377-1381.
65. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. J Surg Oncol. 1983; 24:83-87.
66. Hawk E, Lubet R, Limburg P. Chemoprevention in hereditary colorectal cancer syndromes. Cancer 1999; 86:2551-2563.
67. Steinbach G, Lynch PM, Phillips RK. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000; 342:1946-1952.
68. Hixson LJ, Earnest DL, Fennerty MB. NSAID effect on sporadic colon polyps. Am J Gastroenterol 1993; 88:1652-1656.
69. Ladenheim J, Garcia G, Titzer D. Effect of sulindac on sporadic colonic polyps. Gastroenterology 1995; 108:1083-1087.
70. Matsuhashi N, Nakajima A, Fukushima Y. Effects of sulindac on sporadic colorectal adenomatous polyps. Gut 1997; 40:344-349.
71. Gann PH, Manson JE, Glynn RJ. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst. 1993; 85:1220-1224.
72. Hawk ET, Viner JL, Anderson WF. Development of cyclooxygen inhibitors as cancer chemopreventives, in Perry MC (ed): American Society of Clinical Oncology Educational Book Baltimore, Lippincott Williams & Wilkins, 2001, 28-37.
73. Rubio CA. Antitumoral activity of indomethacin on experimental esophageal tumors. J Natl Cancer Inst. 1984; 72:705-707.
74. Fischer SM, Lo HH, Gordon GB. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol Carcinog 1999; 25:231-240.
75. Rivers JK, McLean DI. An open study to assess the efficacy and safety of topical 3% diclofenac in a 2.5% hyaluronic acid gel for the treatment of actinic keratoses. Arch Dermatol 1997; 133:1239-1242.
76. Grubbs CJ, Lubet RA, Koki AT. Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. Cancer Res 2000; 60:5599-5602.
77. Moore RJ, Zweifel BS, Heuvelman DM. Enhanced antitumor activity by co-administration of celecoxib and the chemotherapeutic agents cyclophosphamide and 5-FU. Proc Am Assoc Cancer Res 2000; 41:409.
78. Milas L, Kishi K, Hunter N. Enhancement of tumor response to gamma-radiation by an inhibitor of cyclooxygenase-2 enzyme. J Natl Cancer Inst. 1999; 91:1501-1504.
79. Subbaramaiah K, Norton L, Gerald W. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer.

Evidence for involvement of AP-1 and PEA3. J Biol Chem. In press.
80. Harris RE, Robertson FM, Abou-Issa HM. Genetic induction and upregulation of cyclooxygenase (COX) and aromatase (CYP19): An extension of the dietary fat hypothesis of breast cancer. Med Hypotheses 1999; 52:291-292.
81. Moos PJ, Fitzpatrick FA. Taxane-mediated gene induction is independent of microtubule stabilization: Induction of transcription regulators and enzymes that modulate inflammation and apoptosis. Proc Natl Acad Sci USA 1998; 95:3896-3901.
82. Moos PJ, Muskardin DT, Fitzpatrick FA. Effect of taxol and taxotere on gene expression in macrophages: Induction of the prostaglandin H synthase-2 isoenzyme. J Immunol. 1999; 162:467-473.
83. Subbaramaiah K, Hart JC, Norton L. Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 and p38 mitogen-activated protein kinase pathways. J Biol Chem. 2000; 275:14838-14845.