REVIEW

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Role of COX-2 selective inhibitors for prevention and treatment of cancer

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Cyclooxygenase-2 (COX-2) is an enzyme induced by inflammatory and mitogenic stimuli and results in enhanced synthesis of PGs in inflamed and neoplastic tissues. It is associated with cell proliferation and growth, in various cancerous conditions. We review the potential mechanisms of cancer reduction with COX-2 inhibitors and the preclinical evidence suggesting their effectiveness. Results of our study show that COX-2 is a regulatory factor for a number of pathways that can result in cancer. COX-2 makes cells resistant to apoptosis and promote angiogenesis, metastasis and cancer cell cycle by controlling number of targets. We found that, COX-2 selective inhibitors (like celecoxib and NS-398) can suppress the cancer both by COX-2 dependent and COX-2 independent pathways. COX-2 inhibitors can also produce synergic effects when used with other anti-cancer therapies. Thus, it is concluded that COX-2 selective inhibitors may be promising agents for prevention and treatment of cancer.

1. Introduction

Cyclooxygenase or prostaglandin endoperoxidase synthase (COX) is an enzyme that catalyzes two sequential reactions involved in the formation of prostaglandins (PGs) from arachidonic acid and is an important chemical mediator for inflammation. COX in its initial reaction catalyses the insertion of molecular oxygen into arachidonic acid to form the unstable intermediate PGG_2, which is rapidly converted to PGH_2 by the peroxidase activity of COX. Specific isomerases then convert PGH_2 into a series of biologically active PGs and thromboxane-A_2. COX is found in two isoforms: COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and appears to be responsible for the production of PGs that control normal physiological functions such as regulation of renal blood flow and maintenance of the gastric mucosa (Smith et al. 2000). By contrast, COX-2 is not detected in most normal tissues. However, it is induced by mitogenic and inflammatory stimuli, which results in enhanced synthesis of PGs in inflamed and neoplastic tissues (Subbaramaiah et al. 1996; Wadleigh et al. 2000; Zweifel et al. 2002). COX-2 is a dimeric molecule which consists of three domains: an N-terminal epidermal growth factor (EGF) domain, a membrane binding domain, the C-terminal catalytic domain with haem, containing the cyclooxygenase and peroxidase active sites.
A variety of preclinical studies have investigated the role of COX-2 in carcinogenesis (Hu et al. 2004; Wun et al. 2004). Tumor formation and growth are reduced in animals that are treated with COX-2 inhibitors or genetically engineered to be COX-2-deficient. COX-2 inhibitors diminish the metastatic potential of tumor cells. The combination of nonselective nonsteroidal anti-inflammatory drugs or selective COX-2 inhibitors with drugs that target the oncogenic pathways may also boost antitumor activity. Moreover, selective COX-2 inhibitors can augment the efficacy of traditional cytotoxic chemotherapy or radiotherapy. Based on these findings many clinical trials (Altorki et al. 2004; Pruthi et al. 2004) assess the potential efficacy of COX-2 inhibitor as anticancer agents. Here we focus on the rationale for using selective COX-2 inhibitors as useful addition to the arsenal of anticancer therapies.

2. Regulation of COX-2 expression

From a very beginning, COX enzyme has found its role in inflammation. In recent years, overexpression of COX-2 has been implicated in the progression of cancer (Hussain et al. 2003; Miguel et al. 1999). Aberrant or increased expression of COX-2 has been found in most of the cancers of the body sites like colorectal, lungs, breast, gastric, pancreatic and esophageal cancer. This over expression of COX-2 appears to be a consequence of both increased transcription and enhanced mRNA stability (Shao 2000; Dixon et al. 2000).
Number of factors can regulate the COX-2 expression by regulating COX promoter. These include oncogenes, growth factors, cytokines and tumor promoters that may stimulate COX-2 transcription via protein kinase C (PKC) and RAS mediated signaling (Subbaramaiah et al. 2000, 2002; Mestre et al. 1997) (Fig. 1). In colorectal cancer COX-2 expression is found to be upregulated by interleukin-1β (IL-1β) via multiple pathways. These pathways include the Erk $^1/_2$ (extracellular regulated kinase), JNK (cJun NH_2 terminal kinases) and p38 MAPK (mitogen ac-

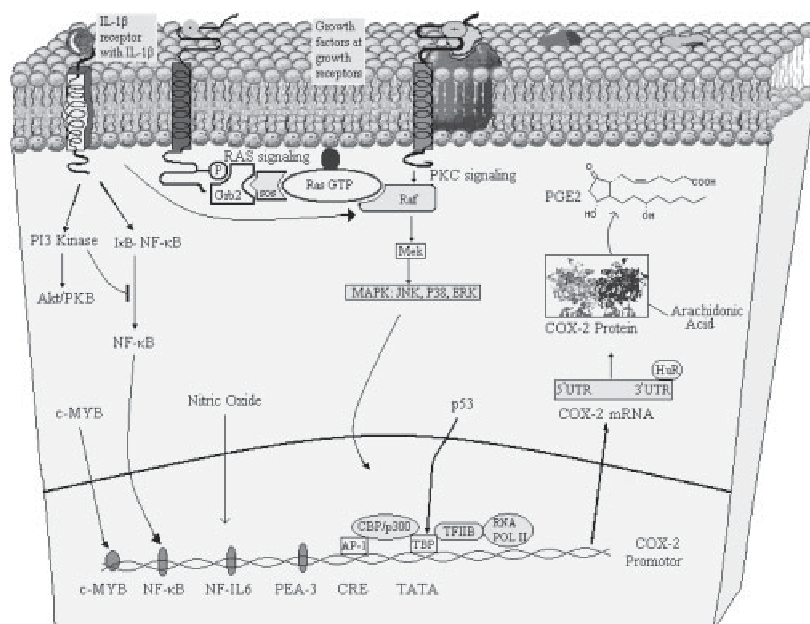

Fig. 1:
Transcriptional and post transcriptional regulation of COX-2 enzyme

tivating protein kinases). In addition, IL-1β also activates PI3k/AKt and NF-κB pathways regulating the COX-2 expression (Liu et al. 2003). It is demonstrated that NF-κB is involved in COX-2 induction by IL-1β. Depending on the stimulus and cell types, a variety of transcription factors including activator protein-1 (AP1), Nuclear factor interleukin-6 (NF-IL6), Nuclear Factor-Kappa B (NF-κB), NF of activated T-cells (NFAT) and polymavirus enhancer activator 3 (PEA3) can modulate the transcription of COX-2 (Subbaramaiah et al. 2002; Smith et al. 2000). Binding of AP-1 and PEA3 on COX-2 promotor is enhanced by HER-2/neu (a transmembrane receptor for epidermal growth factor) by stimulation of Ras-Raf-MAPK signaling transduction pathway (Subbaramaiah et al. 2002). HER-2/neu also uses Akt to increase COX-2 expression (Simeone et al. 2004). NF-κB mediated induction of COX-2 is down regulated by an intestine specific tumor suppressor gene CDX2 (Kim et al. 2004). Recently, the histone acetyl transferase activity of CREB-binding protein/p300 co activator complex was found to be important for AP1 mediated induction of COX-2 (Deng et al. 2004; Subbaramaiah et al. 2002).

There is growing evidence that posttranscriptional mechanisms also determine COX-2 levels in neoplastic tissues. The 3′-untranslated region (UTR) of COX-2 in mRNA contains a series of shaw-kamen sequences (AUUUA, also known as AU-enriched elements) that confers a message of instability (Cok et al. 2001; Sheng et al. 2000). Oncogenes, cytokines, growth factors and tumor promoters also induce COX-2 by enhancing mRNA stability in addition to stimulating transcription. HuR (an RNA binding protein) binding to 3′UTR was also found to increase message stability in colon cancer.

3. Contribution of COX-2 in cancer

In recent years, overexpression of COX-2 has been associated with the progression of cancer and has been found in most of the cancers of the body sites. Compelling evidence from various studies indicate that COX-2 upregulation is one of the key steps in carcinogenesis (Ferrandina et al. 2002; Subbarayan et al. 2001; Joo et al. 2002). Numerous pharmacological studies indicate that COX-2 is a therapeutic target, which supports the concept that selective COX-2 inhibitors might be useful for preventing cancer (Reddy et al. 2000; Evans et al. 2003; Gupta et al. 2004). The major regulatory effect of COX-2 in carcinogenesis is produced via increased levels of prostaglandin E_2 (PGE_2) (Zweifel et al. 2002). Thus, various studies have confirmed the contribution of the COX-2 enzyme to tumorigenesis through regulation of angiogenesis, induction of metastasis, inhibition of apoptosis, and regulation of cell cycle.

3.1. Regulation of angiogenesis

Angiogenesis is an important factor in tumor development. For exponential tumor growth, tissues must receive increased nutrient and oxygen supply. But blood vessels do not proliferate beyond $1-2\ mm^3$ tissue layer. This is made possible by neovascularization thereby increasing vascular supply to the newly forming tissues (Folkman 1990). The onset of angiogenesis also contributes to metastasis. It is found that COX-2 is involved in regulation of angiogenesis in cancer cells (Wang and Dubois 2004; Chu et al. 2003; Yu et al. 2003; Tsuji et al. 1998). Various studies explain that increased COX-2 expression in cancer cells stimulates angiogenesis through prostaglandin E_2 (PGE_2) production. PGE_2 production results in induction of Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) mRNA expression (Fig. 2). The induction of VEGF seems to occur through activation PKA pathway and bFGF is induced by PKA and PKC activation (Cheng et al. 1998).

Selective COX-2 inhibitors are found to inhibit angiogenesis by decreasing the VEGF expression (O'Donoghue et al. 2003). An investigation (Chu et al. 2003) conducted to test the potential involvement of COX-2 pathway in regulation of angiogenesis and growth in pancreatic cancer shows that pretreatment of BxPC-3 cells (a COX-2 positive) with NS-398 dramatically decreases angiogenic responses of endothelial cells. NS398 had no effect on AsPC 1 (a COX-2 negative human pancreatic cell line) cell growth. A pervious study (Molina et al. 1999) also shows the very similar result suggesting the use of sulindac sulfide and NS398 in the chemoprevention and therapy of pancreatic carcinoma.

Preclinical studies have shown that celecoxib is a potent antiangiogenic agent *in vitro* and *in vivo* (Leahy et al. 2002). Oral celecoxib (30 mg/kg/day) inhibited angiogenesis by 79% in a rat model of bFGF-induced corneal angiogenesis, and reduced corneal levels of PGE_2 and TXB_2 by 79% and 68%, respectively. Celecoxib can also inhibit angiogenesis via a COX-2 independent mechanism. Impaired VEGF gene expression and decreased angiogenesis result from celecoxib induced interference with DNA binding of the Sp1 transcription factor (Wei et al. 2003). Celecoxib also has been reported to increase serum levels of the endogenous angiogenesis inhibitor endostatin while decreasing the release of VEGF by platelets (Ma et al. 2002), thus altering the balance of angiogenesis regulation in favour of inhibition.

Rofecoxib also has been shown to inhibit angiogenesis in a number of *in vivo* systems. Administration of rofecoxib blocks the production of bFGF and reduces wound-healing angiogenesis in experimental gastric ulcers (Guo et al. 2002). In a mouse model of retinopathy, rofecoxib inhibited neovascularisation in COX-2 expressing retinal vessels (Wilkinson et al. 2003). In preclinical studies, celecoxib and rofecoxib have shown to generate additive or synergistic benefit in combination with standard chemotherapy agents (Gately and Kerbel 2001) or radiation therapy (Kishi et al. 2000; Petersen et al. 2000).

3.2. Inhibition of metastasis

Metastasis is the process of migration of tumor cells to other parts of the body. Highly aggressive tumors rapidly outgrow their blood supply, leaving the cells starved of oxygen – a condition known as hypoxia. Tumor cells adapt to hypoxia by increasing their synthesis of a protein named HIF (Hypoxia Induced Factor) which in turn binds to and activates several genes like VEGF, EPO, *c-Met* and *CXCR4* (Fig. 2). VEGF and erythropoietin (EPO) increase oxygen supply to the tissues. *c-Met* enhances cell motility and invasion and therefore tumor cells are stimulated to move away from site of hypoxia. *CXCR4*, a chemokine receptor governs organ-specific metastasis by interacting with matching chemokines in target organs. The von Hippel-Lindau tumor suppressor gene (pVHL) negatively regulates *CXCR4* expression owing to its capacity to target the hypoxia-inducible factor for degradation under normoxic conditions. This process is suppressed under hypoxic conditions and in tumor derived mutants of pVHL resulting in HIF. *CXCR4* stimulates migration and enables tumor cells to home in on specific distant organs (Bernards 2003; Staller 2003).

In many conditions, COX-2 expression is correlated with HIF-1 activation (Liu et al. 2002). It is stated that in cancerous conditions, increased COX-2 expression induces translocation of HIF-1α protein to the nucleus through PGE_2 mediated activation of EP2 and EP4 receptors. EP receptor activation results in phosphorylation of HIF-1α

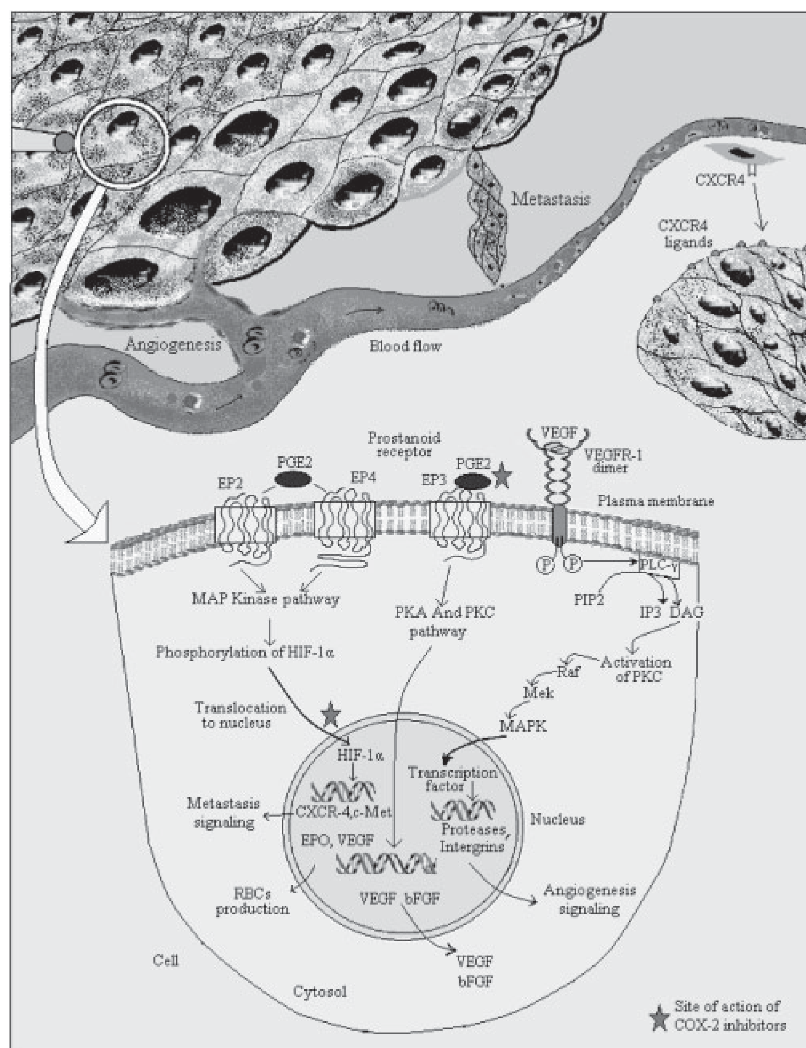

Fig. 2:
Regulation of angiogenesis and metastasis

protein through MAP kinase pathway. This results in nucleus translocation and stabilization of the protein. Selective COX-2 inhibitors like meloxicam and NS-398 and other non selective NSAIDs are found to inhibit hypoxia induced VEGF expression and HIF-1α accumulation and upregulated VHL expression (Jones et al. 2002; Palayoor 2003). All these studies shows that PGE_2 production via COX-2 catalyzed pathway plays a critical role in HIF-1α regulation by hypoxia and imply that COX-2 inhibitors can prevent hypoxic induction of HIF-mediated gene transcription in cancerous cells. Recent studies by Yao et al. (Yao et al. 2004) have shown that the expression and activity of COX-2 appears to be associated with the proliferative and invasive properties of colorectal cancer (CRC). Cyclooxigenase inhibition by NS-398 suppresses tumor cell growth and invasion/migration, and retards the formation of liver metastasis in a mouse CRC model, via multiple cellular and molecular mechanisms.

3.3. Induction of apoptosis

Apoptosis or programmed cell death is a group of events that proceed in a systematic order and selectively removes unwanted extra or damaged cells. Apoptosis allows the organism to tightly control cell number and tissue size and to protect itself from rogue cells that threaten homeostasis (Hengartner 2000).

Selective COX-2 inhibitors have been demonstrated to induce apoptosis in variety of cancers cells, including those of colon (Li et al. 2001a), stomach (Li et al. 2001b), prostate (Song et al. 2002) and breast (Haris et al. 2000). These observations are consistent with the COX-2 inhibitor being a chemopreventive agent that increases the susceptibility of cancer cells to apoptosis. It is well documented that COX-2 is constitutively overexpressed in many types of human cancers and that decreased prostaglandin E_2 production as a result of COX-2 inhibition is associated with the modulation of various pro- and anti-apoptotic factors, such as Bcl_2 (Sheng et al. 1998) prostrate apoptosis-response gene (Par-4) (Zhang 2000) and caspase-3 (McGinty et al. 2000) (Fig. 3).

In addition, knockout of the COX-2 gene suppresses tumerigenisis in mice that have a genetic predisposition to form polyps (Oshima et al. 1996). Recently, the U.S. Food and Drug Administration approved the use of the COX-2 inhibitor celecoxib for the adjuvant treatment of familial adenomatous polyposis, an inherited syndrome that predisposes individuals to colon cancer.

In addition, celecoxib has also been tested in numerous clinical trials (Hawk et al. 2002) for its chemopreventive effect on a variety of epithelial malignancies including colon, esophagous, skin and bladder cancers. However, an expanding body of evidence suggests that COX-2 inhibition may not play a role in NSAID mediated apoptic cell death (Marx 2001). For example, sulindac sulfide and sulindac sulfone, which are metabolites of the NSAID sulindac, have been reported to mediate apotosis in cancer cells via the inhibition of cyclic GMP phosphodiesterase (Thompson et al. 1997; Lim et al. 1999), which is a COX-2 independent mechanism (Hann 2001). A tetracycline-inducible antisense COX-2 expression plasmid demonstrated that the sensitivity of prostate cancer cells to COX-2 inhibitor-induced apoptosis is independent of the expression status of COX-2 (Song 2002). It has also been reported that celocoxib induces apop-

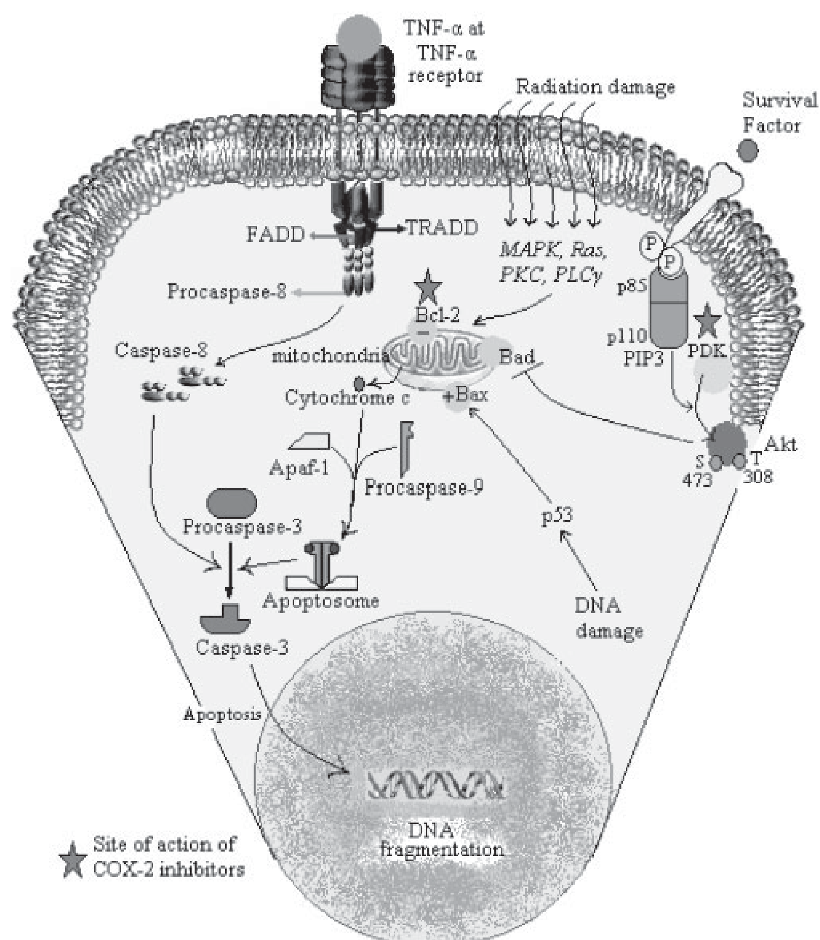

Fig. 3:
Regulation of apoptosis by COX-2 and its inhibitors

tosis in prostate cancer cells by interacting with multiple signaling targets, including the serine/threonine kinase Akt, extracellular signal-regulated kinase 2 (ERK2), and endoplasmic reticulum Ca^{2+}-ATPases (Hsu et al. 2000; Johnson et al. 2001). Disruption of these signaling pathways results in the loss of regulation of cellular functions that govern cell growth and survival, leading to rapid apoptotic death.

COX-2 overexpression has been shown to upregulate Bcl_2 expression with an associated decrease in apoptosis (Tsuji and Dubois 1995). The human prostrate carcinoma LNCaP cells, which overexpress COX-2, exhibit apoptosis induction and Bcl_2 downmodulation when treated with NS 398, a selective COX-2 inhibitor (Liu 1998). Inhibition of COX-2 by celecoxib has been shown to induce apoptosis in both androgen-responsive LNCaP and androgen-unresponsive PC-3 cells by blocking Akt phosphorylation, which is independent of Bcl_2 (Hsu et al. 2000). Furthermore a recent study by Lai et al. (2003) has shown that P^{185neu} tyrosine kinase inhibitor emodin (Zhang et al. 1999) in combination with the COX-2 inhibitor celecoxib (Reddy et al. 2000) acts synergistically to suppress the growth of both rat C611B cholarogiocarcinoma (chc) cells and neu-transformed rat liver epithelial stem-like cells (WBneu cells) in culture. They indicated that this effect is the result of a synergistic action to enhance apoptosis through a mechanism involving inhibition of Akt activation leading to increased activation of caspase-mediated apoptosis. Thus the results show that celecoxib is acting independently of its ability to inhibit COX-2 activity in suppressing growth of C611B and WBnew cells *in vitro*.

3.4. Cell cycle regulation

In the last few years, COX-2 enzyme has shown its importance as a cell cycle regulator in various cancer cells. COX-2 inhibitors can effectively produce cell cycle arrest by regulating G_1 and S phases of the cell cycle (Fig. 4). Mammalian cells are controlled by a number of extracellular growth factors and intracellularly triggered controls, to undergo proliferation. In cancer, the control of proliferation is deranged due to cell-cycle disregulation (Pardee 1989). Normally, the transition between different cell cycle states is regulated at various checkpoints. These check points are regulated by a family of protein kinases, the cyclin dependent kinases (CDKs) and their obligate activating partners, the cyclins (Hunter and Pines 1994). Cyclins are the result of the transcription and translation processes and their abundance varies during specific phases of cell cycle (Koepp et al. 1999).

Studies by Toyoshima et al. (2002) showed that growth inhibition of NA, a cancer cell line established from a patient with SCC of the tongue, by NS398 a COX-2 inhibitor was associated with Go/G1 cell cycle arrest. Western blot analysis showed that NS398 upregulated p21 protein, a specific inhibitor of CDKs, in NA cells. Moreover, growth inhibition induced by NS398 was reduced in p21 antisense treated NA cells compared to untreated NA cells. Thus, the accumulation in G0/G1 by NS398 might be mediated by up-regulation of p21. Nakanishi et al. (2001) have shown that NS398 and nabumetone suppressed the proliferation of two leukemia cell lines U-937 and ML-1 cells by inducing a G0/G1 cell-cycle arrest. Cell-cycle arrest induced by these COX-2 inhibitors was not associated with an upregulation of the cyclin-dependent kinase inhibitors. COX-2 inhibitors also inhibited the differentiation of these cells induced by differentiation-inducing factors (DIFs) such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and retinoic acid (RA). Treatment with NS-398 did not suppress the levels of PG produced by these cells. Although COX-2 antisense oligonucleotides showed a similar inhibitory effect on these cells, their inhibitory effect was smaller than that of NS-398. These results suggested that COX-2 inhibitors may suppress the proliferation and differentiation of leukemia cells both via COX-2 dependent and independent pathways.

Hang et al. (2000) have reported that the expression of p27 is increased in lung cancer cells after treatment with COX-2 inhibitor NS-398, suggesting that cdk inhibitors may be potential targets for COX-2 inhibitor mediated in-

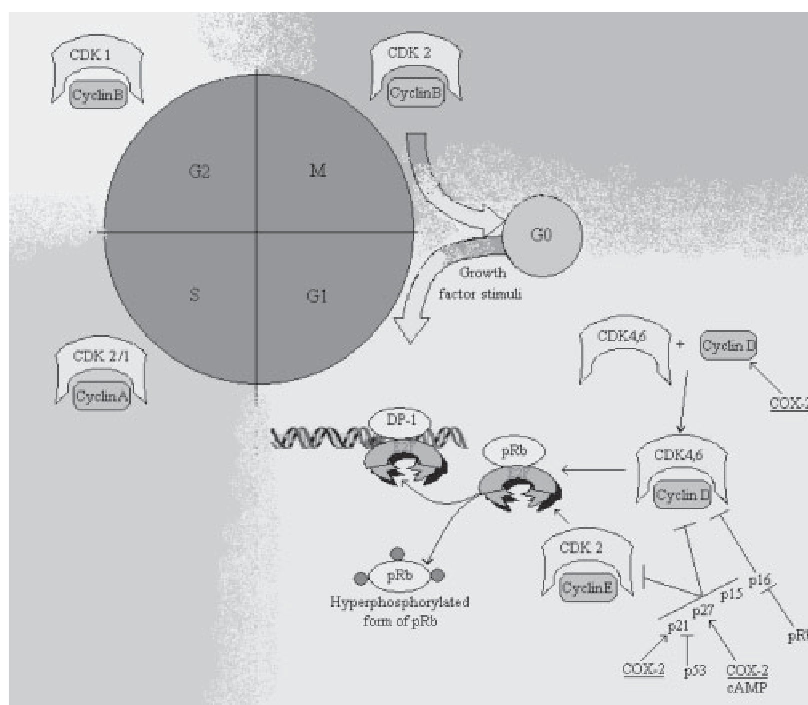

Fig. 4:
Cancer cell cycle and effect of COX-2 on G1 phase

hibition of tumor growth. Studies have shown that celecoxib inhibits the growth of several tumor cell types (Steinbach 2000; Grosch 2001). Recently Han et al. (2004) performed *in vitro* studies and showed a direct role of COX-2 in cholangiocarcinoma cell growth by overexpression and antisense depletion of COX-2. They showed that celecoxib treatment significantly increased the p21 and p27 protein level in a dose and time dependent fashion in human cholangiocarcinoma cells whereas the protein levels of p18 and GADD45 were not altered. The cells treated with celecoxib showed increased binding of p21 and p27 to cdk2 kinase complex and decreased cdk2 kinase activity but no change in cdk2 and cyclin E protein levels. Consistent with these findings, flow cytometric analysis showed that celecoxib induced G1-S arrest with no significant effect on G2-M transition.
These results provided a link between p21/p27 and celecoxib-mediated inhibition of intrahepatic cholangiocarcinoma cell growth. Their findings provided evidence for the involvement of a COX-2 independent mechanism in celecoxib mediated inhibition of human intrahepatic cholangiocarcinoma cell growth. The fact that overexpression or antisense depletion of COX-2 failed to alter the levels of p21 and p27 indicate the existence of a COX-2 independent effect. Thus, although celecoxib potentially inhibits human cholangiocarcinoma cell growth, its antitumor effect is mediated, at least in part, through mechanisms independent of COX-2 inhibition.

4. Effect of COX-2 inhibitors in combination therapy

Recently, a combination of more than one target has emerged as an approach for providing an effective cancer prevention with fewer side effects. Preclinical studies show that COX-2 selective inhibitors can synergize the effectiveness of other therapeutic approaches such as radiation therapy (Komaki et al. 2004; Nakata et al. 2004), photodynamic therapy (Hendrick et al. 2003) and other chemotherapeutic agents (De Long 2003; Badawi et al. 2004). Preclinical studies explain that the radiation can elevate intratumoral levels of COX-2 protein and its products particularly PGE_2 (Terakado et al. 2004; Davis et al. 2004). PGs are found to exert a protective role in radiation therapy when administered before irradiation. In this concern, selective COX-2 inhibitors e.g. celecoxib increase the radiosensitivity for radiotherapy and synergize the control over tumor growth.
In another approach simultaneous targeting of COX-2 and PPARγ (Peroxisome Proliferator Activated Receptor γ) has been correlated for inhibiting mammary cancer development. COX-2 inhibitors and PPARγ agonists coordinately mediate their anticancer effect via both COX-2 dependent (inhibition of COX-2, activation of PPARγ and modulation of PG synthesis) and COX independent (induction of proapoptotic factors and inhibition of cell proliferation) pathways (Badawi et al. 2004). Similarly, the use of COX-2 inhibitors can enhance the efficacy of immunotherapy (Delong et al. 2003). IFN beta therapy combined with COX-2 inhibition was associated with an increased number of T-cells within tumors and resulted in cure of small tumors, significant inhibition of the growth of large established tumors and inhibition of growth of metastatic tumor foci after surgical debulking. Combination of Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitor with an COX-2 inhibitor also caused a cooperative antitumor effect in breast cancer cells (Rosato et al. 2003).

5. Conclusions

The COX-2 enzyme is a key regulatory factor in various cancerous conditions and its inhibition provides an important target for cancer chemotherapy. Overexpression of COX-2 is strongly implicated in regulation of angiogenesis through induction of Vascular Endothelial Growth Factor (VEGF) and translocation of HIF-1α protein to nucleus through PGE_2 resulting in metastasis. Upregulation of COX-2 directly resists apoptosis by increasing the expression of proapoptotic Bcl-2 proteins and inhibiting cytochrome c release from mitochondria. COX-2 enzyme also produces an inducing effect on cell proliferation by controlling G1 and S phase cyclins.
Selective COX-2 inhibitors commonly reduce the growth rate of established tumors. These drugs are reported to suppress the cancer both by inhibiting the COX-2 activity and by interacting with non-COX-2 targets. Preclinical studies show that co-treatment with COX-2 inhibitors augments the antitumor effects of chemotherapy, radiation and photodynamic therapy. Thus, these studies suggest that use of selective COX-2 inhibitors may play an important future role in both the treatment and prevention of cancer.

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References

Altorki N (2004) COX-2: a target for prevention and treatment of esophageal cancer. J Surg Res 117: 114–120.
Badawi AF, Eldeen MB, Liu Y, Ross EA, Badar MZ (2004) Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor gamma. Cancer Res 64: 1181–1189.
Bernards R (2003) Cues for migration. Nature 425: 247–248.
Cheng T, Cao W, Wen R, Steinberg RH, LaVail MM (1998) Prostaglandin E2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat muller cells. Invest Ophthalmol Vis Sci 39: 581–591.
Chu J, Lloyd FL, Trifan OC, Knapp B, Rizzo MT (2003) Potential involvement of the cyclooxygenase-2 pathway in the regulation of tumor-associated angiogenesis and growth in pancreatic cancer. Molecular Can. Ther 2: 1–7.
Cok SJ, Morrison AR (2001) The 3′-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem 276: 23179–23185.
Davis TW, O'Neal JM, Pagel MD, Zweifel BS, Mehta PP, Heuvelman DM, Masferrer JL (2004) Synergy between celecoxib and radiotherapy results from inhibition of cyclooxygenase-2-derived prostaglandin E2, a survival factor for tumor and associated vasculature. Cancer Res 64: 279–285.
De Long P, Tanaka T, Kruklitis R, Henry AC, Kapoor V, Kaiser LR, Sterman DH, Albelda SM (2003) Use of cyclooxygenase-2 inhibition to enhance the efficacy of immunotherapy. Cancer Res 63: 7845–7852.
Deng WG, Zhu Y, Wu KK (2004) Role of p300 and PCAF in regulating cyclooxygenase-2 promoter activation by inflammatory mediators. Blood 103: 2135–2142.
Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM (2000) Post-transcriptional control of cyclooxygenase-2 gene expression. J Biol Chem 275: 11750–11757.
Evans JF (2003) Rofecoxib (Vioxx), a specific cyclooxygenase-2 inhibitor, is chemopreventive in a mouse model of colon cancer. Am J Clin Oncol 26: S62-S65.
Ferrandina G, Legge F, Ranelletti FO, Zannoni GF, Maggiano N, Evangelisti A, Mancuso S, Scambia G, Lauriola L (2002) Cyclooxygenase-2 expression in endometrial carcinoma. Cancer 95: 801–807.
Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82: 4–6.
Gately S, Kerbel R (2001) Antiangiogenic scheduling of lower dose cancer chemotherapy. Cancer J 7: 424–436.
Grosch S, Tegeder I, Niederberger E, Brautigam L, Geisslinger G (2001) COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. FASEB J 15: 2742–2744.
Guo JS, Cho CH, Lam ES (2002) Antiangiogenic effect of a highly selective cyclooxygenase-2 inhibitor on gastric ulcer healing in rats. Toxicol Appl Pharmacol 183: 44–45.

Gupta S, Adhami VM, Subbarayan M, MacLennan GT, Lewin JS, Hafeli UO, Fu P, Mukhtar H (2004) Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. Cancer Res 64: 3334–3343.

Han C, Leng J, Demetris J, Wu T (2004) Cyclooxygenase-2 promotes human cholangiocarcinoma growth: Evidence for cyclooxygenase-2-independent mechanism in celecoxib-mediated induction of $p21^{waf1/cip1}$ and $p27^{kip1}$ and cell cycle arrest. Cancer Res 64: 1369–137.

Hann C (2001) Sulindac and its derivatives: a novel class of anticancer agents. Curr Opin Investig Drugs 2: 677–683.

Haris RE, Alshafie GA, Abou-Issa H, Sibert K (2000) Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase inhibitor. Cancer Res 60: 2101–2103.

Hawk ET, Viner JL, Dannenberg A, DuBois RN (2002) COX-2 in cancer – a player that's defining the rules. J Natl Cancer Inst 94: 545–546.

Hendrickx N, Volanti C, Moens U, Seternes OM, de Witte P, Vandenheede JR, Piette J, Agostinis P (2003) Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. J Biol Chem 278: 52231–52239.

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407: 770–776.

Hsu AL, Ching TT, Wang DS, Song X, Rngnekar VM, Chen CS (2000) The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostrate cancer cells independently of Bcl-2. J Biol Chem 275: 11397–11403.

Hu PJ, Yu J, Zeng ZR, Leung WK, Lin HL, Tang BD, Bai AH, Sung JJ (2004) Chemoprevention of gastric cancer by celecoxib in rats. Gut 53: 195–200.

Hung WC, Chang HC, Pan MR, Lee TH, Chuang LY (2000) Induction of p27 as a mechanism underlying NS398-induced growth inhibition in human lung cancer cells. Molec Pharmacol 58: 1398–1403.

Hunter ET, Pines J (1994) Cyclins and cancer 2: Cyclin D and CDK inhibitors come of age. Cell 79: 573–582.

Hussain T, Gupta S, Mukhtar H (2003) Cyclooxygenase-2 and prostate carcinogenesis. Cancer Lett 191: 125–135.

Johnson AJ, Song X, Hsu A, Chen C (2001) Apoptosis signaling pathways mediated by cyclooxygenase-2 inhibitors in prostrate cancer cells. Adv Enzyme Regul 41: 221–235.

Jones MK, Szabo IL, Kawanaka H, Husain SS, Tarnawshi AS (2002) Von Hippel Lindau tumor suppressor and HIF-1α: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. FASEB 16: 264–266.

Joo YE, Kim HS, Min SW, Lee WS, Park CH, Park CS, Choi SK, Rew JS, Kim SJ (2002) Expression of cyclooxygenase-2 protein in colorectal carcinomas. Int J Gastrointest Cancer 31: 1–3.

Kim SP, Park JW, Lee SH, Lim JH, Jang BC, Lee SH, Jang IH, Freund JN, Suh SI, Mun KC, Song DK, Ha EM, Lee WJ, Kwon TK (2004) Homeodomain protein CDX2 regulates COX-2 expression in colorectal cancer. Biochem Biophys Res Commun 315: 93–99.

Komaki R, Liao Z, Milas L (2004) Improvement strategies for molecular targeting: cyclooxygenase-2 inhibitors as radiosensitizers for non-small cell lung cancer. Semin Oncol 31: 47–53.

Leahy KM, Omberg RL, Wang Y (2002) Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in angoigenic endothelial cells *in vivo*. Cancer Res 62: 625–631.

Lai GH, Zhang Z, Sirica AE (2003) Celecoxib acts in a cyclooxygenase-2-independent manner and in synergy with emodin to suppress rat cholangiocarcinoma growth *in vitro* through a mechanism involving enhanced Akt inactivation and increased activation of caspases-9 and -3. Mol Cancer Ther 2: 265–271.

Li M, Wu X, Xu XC (2001) Induction of apoptosis in colon cancer cells by cyclooxygenase-2 inhibitor NS398 through a cytochrome c-dependent pathway. Clinical Cancer Res 7: 1010–1016.

Li M, Wu X, Xu XC (2001) Induction of apoptosis by cyclo-oxygenase-2 inhibitor NS398 through a cytochrome C dependent pathway in esophageal cancer cells. Int J Cancer 93: 218–223.

Lim JT, Piazza GA, Han EK, Delohery TM, Li H , Finn TS (1999) Sulindac derivatives inhibit growth and induce apoptosis in human prostrate cancer cell lines. Biochem Pharmacol 58: 1097–1017.

Liu W, Reinmuth N, Stoeltzing O, Parikh AA, Tellez C, Williams S, Jung YD, Fan F, Takeda A, Akagi M, Bar-Eli M, Gallick GE, Ellis M (2003) Cyclooxygenase-2 is up-regulated by interleukin-1 beta in human colorectal cancer cells via multiple signaling pathways. Cancer Res 63: 3632–3636.

Liu XH, Kirschenbaum A, Lu M, Yao S, Dosoretz A, Holland JF, Levine AC (2002) Prostaglandin E_2 induces hypoxia-inducible factor-1α stabilization and nuclear localization in a human prostate cancer cell line. J Biol Chem 277: 50081–50086.

Liu XH, Yao S, Kirschenbaum A, Levine AC (1998) NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCap cells. Cancer Res 58: 4245–4249.

Ma L, del Soldato P, Wallace JL (2002) Divergent effects of new cyclooxygenase inhibitors on gastric ulcers healing: Shifting the angiogenic balance. Proc Natl Acad Sci USA 99: 13243–13247.

Marx J (2001) Anti-inflammatories inhibit cancer growth – but how? Science 291: 581–582.

McGinty A, Chang YW, Sorokin A, Bokemeyer D, Dunn MJ (2000) Cyclooxygenase-2-expression inhibits trophic withdrawl apoptosis in nerve growth factor-differentiated PC12 cells. J Biol Chem 275: 12095–12101.

Mestre JR, Subbaramaiah K, Sacks PG, Schantz SP, Tanabe T, Inoue H, Dannenberg AJ (1997) Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squmous carcinoma cells. Cancer Res 57: 2890–2895.

Miguel AM, Marta SA, Michael GL, Marsha LF, Frank AS (1999) Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 59: 4356–4362.

Molina MA, Arnau MS, Lemoine MG, Frazier ML, Sinicrope FA (1999) Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 59: 4356–4362.

Nakanishi Y, Kamijo R, Takizawa K, Hatori M, Nagumo M (2001) Inhibitors of cyclooxygenase-2 (COX-2) suppressed the proliferation and differentiation of human leukaemia cell lines. Eur J Cancer 37: 1570–1578.

Nakata E, Mason KA, Hunter N, Husain A, Raju U, Liao Z, Ang KK, Milas L (2004) Potentiation of tumor response to radiation or chemoradiation by selective cyclooxygenase-2 enzyme inhibitors. Int J Radiat Oncol Biol Phys 58: 369–375.

O'Donoghue GT, Roche-Nagle G, Connolly EM, Harmey J, Bouchier-Hayes DJ (2003) Selective COX-2 inhibition attenuates the perioperative increase of the tumor enhancing pro-angiogenic cytokine VEGF in human breast cancer patients. J Surg Res 114: 243.

Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase2 (COX-2). Cell 87: 803–809.

Palayoor ST, Tofilon PJ, Coleman CN (2003) Ibuprofen-mediated reduction of hypoxia-inducible factors HIF-1α and HIF-2α in prostate cancer cells. Clinical Cancer Res 9: 3150–3157.

Pardee AB (1989) G_1 events and regulation of cell proliferation. Science 246: 603–608.

Petersen C, Petersen S, Milas L (2000) Enhancement of intrinsic tumor cell radiopsensitivity induced by a selective cyclooxygenase-2 inhibitor. Clin Cancer Res 6: 2513–2520.

Pruthi RS, Derksen JE, Moore DA (2004) Pilot study of use of the cyclooxygenase-2 inhibitor celecoxib in recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. BJU Int 93: 275–278.

Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV (2000) Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. Cancer Res 60: 293–297.

Rosato FE, Dicker A, Burd R, Miller S, Lanza-Jacoby S (2003) Combining the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, ZD1839, with a selective cyclooxygenase (COX)-2 inhibitor, SC236, causes a cooperative antitumor effect in breast cancer cells derived from HER2/neu mice. J Surg Res 114: 274.

Shao J (2000) Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. J Biol Chem 275: 33951–33956.

Sheng H, Shao J, Dixon DA, Williams CS, Prescott SM, Dubois RM, Beauchamp RD (2000) Transforming growth factor-β1 enhances Ha-ras induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. J Biol Chem 275: 6628–6635.

Sheng H, Shao J, Morrow JD, DuBois RN (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 58: 362–366.

Simeone AM, Li YJ, Broemeling LD, Johnson MM, Tuna M, Tari AM (2004) Cyclooxygenase-2 is essential for HER2/neu to suppress N-(4-hydroxyphenyl) retinamide apoptotic effects in breast cancer cells. Cancer Res 64: 1224–1228.

Smith WL (2000) Cyclooxygenase: structural, cellular and molecular biology. Annu Rev Biochem 69: 145–182.

Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenase: structural, cellular and molecular biology. Annu Rev Biochem 69: 145–182.

Song X, Lin HP, Johnson AJ, Tseng PH, Yang YT, Kulp SK (2002) Cyclooxygenase-2. Player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostrate cancer cells. J Natl Cancer Inst 4: 585–591.

Staller P, Sulitkova J, Lisztwn J, Moch H, Oakeley EJ, Krek W (2003) Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature 425: 307–311.

Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B (2000) The effect of celecoxib, a cyclooxygenase-2-inhibitor, in familial adenomatous polyposis. N Engl J Med 342: 1946–1952.

Subbaramaiah K, Cole PA, Dannenberg AJ (2002) Retinoids and carnosol suppress cyclooxygenase-2 transcription by CREB-binding protein/p300-dependent and -independent mechanisms. Cancer Res 62: 2522–2530.

Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ (2000) 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 275: 14838–14845.
Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ (2002) Cyclooxygenase-2 in overexpressed in HER-2/neu-positive breast cancer. J Biol Chem 277: 18649–18657.
Subbaramaiah K, Telang N, Ramonetti JT, Araki R, deVito B, Weksler BB, Dannenberg AJ (1996) Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res 56: 4424–4429.
Subbarayan V, Sabichi AL, Lansa N, Lippman SM, Menter DG (2001) Differential expression of cyclooxygenase-2 and its regulation by Tumor Necrosis Factor-α in normal and malignant prostate cells. Cancer Res 61: 2720–2726.
Terakado N, Shintani S, Yano J, Chunnan L, Mihara M, Nakashiro K, Hamakawa H (2004) Overexpression of cyclooxygenase-2 is associated with radioresistance in oral squamous cell carcinoma. Oral Oncol 40: 383–389.
Thompson HJ, Jiang C, Lu J, Mehta RG, Piazza GA, Paranka NS (1997) Sulfone metabolite of sulindac inhibits mammary carcinogenesis. Cancer Res 57: 267–271.
Toyoshima T, Kamijo R, Takizawa K, Sumitani K, Ito D, Nagumo M, (2002) Inhibitor of cyclooxygenase-2 induces cell-cycle arrest in the epithelial cancer cell line via up-regulation of cyclin dependent kinase inhibitor p21. British J Cancer 86: 1150–1156.
Tsuji M, Dubois RN (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 83: 493–501.
Tsuji M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 93: 705–716.
Wadleigh DJ, Reddy ST, Kopp E, Ghosh S, Herschman HR (2000) Transcriptional activation of the cyclooxygenase-2 gene in endotoxin treated RAW 264.7 macrophages. J Biol Chem 275: 6259–6266.
Wang D, Dubois RN (2004) Cyclooxygenase-2: a potential target in breast cancer. Semin Oncol 31: 64–73.
Wei D, Wang L, He Y (2003) Celecoxib inhibits vascular endothelial growth factor expression by targeting Sp1 transcription factor and reduces human pancreatic cancer angiogenesis and metastasis. Proc Am Assoc Cancer Res 44: 633.
Wilkinson-Berka JL, Alousis NS, Kelly DJ (2003) COX-2 inhibition and retinal angiogenesis in a mouse model of retinopathy of prematurity. Invest Ophthalmol Vis Sci 44: 974–979.
Wun T, McKnight H, Tuscano JM (2004) Increased cyclooxygenase-2 (COX-2): a potential role in the pathogenesis of lymphoma. Leuk Res 28: 109–111.
Zhang L, Lau YK, Xia W, Hortobagyi GN, Hung MC (1999) Tyrosine kinase inhibitor emodin suppresses growth of HER-2/neu-over-expressing breast cancer cells in athymic mice and sensitizes these cells to the inhibitory effect of paclitaxel. Clin Cancer Res 5: 343–353.
Zhang Z, DuBois RN (2000) Par-4 a proapoptotic gene is regulated by NSAIDs in human colon carcinoma cells. Gastroenterology 118: 1012–1017.
Zweifel BS, Davis TW, Ornberg R, Masferrer JL (2002) Direct evidence for a role of cyclooxygenase-2 derived prostaglandin E2 in human head and neck xenograft tumors. Cancer Res 62: 6706–6711.