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Cyclooxygenase-2 as a Molecular Target for Cancer Chemopreventive Agents

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ABSTRACT *: Recently, considerable attention has been focused on the role of cyclooxygenase-2 (COX-2) in the carcinogenesis as well as in inflammation. Improperly overexpressed COX-2 has been observed in many types of human cancers and transformed cells in culture. Thus, it is conceivable that targeted inhibition of abnormally or improperly up-regulated COX-2 provides one of the most effective and promising strategies for cancer prevention. A ubiquitous eukaryotic transcription factor, NF-κB is considered to be involved in regulation of COX-2 expression. Furthermore, extracellular-regulated protein kinase and p38 mitogen-activated protein (MAP) kinase appear to be key elements of the intracellular signaling cascades involved in NF-κB activation in response to a wide array of external stimuli. Certain chemopreventive phytochemicals suppress activation of NF-κB by blocking one or more of the MAP kinases, which may contribute to their inhibitory effects on COX-2 induction. One of the plausible mechanisms by which chemopreventive phytochemicals inhibit NF-κB activation involves suppression of degradation of the inhibitory unit IκB, which hampers subsequent translocation of p65, the functionally active subunit of NF-κB.*

Key Words *: Chemoprevention, Cyclooxygenase-2 (COX-2), Prostaglandins, NF-κB, Mitrogen-activated protein kinases*

I. ROLE OF PROSTAGLANDINS IN MALIGNANT TRANSFORMATION

There has been increasing evidence from both epidemiologic and experimental studies, supporting that prostaglandins (PGs) play roles in carcinogenesis as well as inflammation. Elevated levels of PGs have been often observed in various types of human cancers (Bennett *et al.*, 1986; Rigas *et al.*, 1993; Vandereen *et al.*, 1986). In line with this notion, epidemiologic studies have revealed a significant reduction in the risk of colorectal, gastric, esophageal, and breast cancers among people who regularly take nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, compared with those not taking NSAIDs (Giovannucci *et al.*, 1995; Greensberg *et al.*, 1993; Schrenemachers and Everson, 1994; Thun, 1994). Furthermore, the NSAID sulindac (structure shown in Fig. 1) reduces the size and the number of intestinal adenomas in patients with familial adenomatous polyposis (Giardiello *et al.*, 1993; Nugent *et al.*, 1993; Wadell *et al.*, 1989). Chemopreventive effects of NSAIDs have also been confirmed in experimentally induced carcinogenesis studies (Boolbol *et al.*, 1996; Castonguay and Rioux, 1997; Giardiello *et al.*, 1995; McCormic *et al.*, 1985; Perkins and Shklar, 1982; Rao *et al.*, 1995; Reddy *et al.*, 1993; Takahashi *et al.*, 1990). The cancer chemopreventive properties of NSAIDs have been attributed to their inhibition of cyclooxygenase (COX) that catalyzes the rate-limiting step in the conversion of arachidonic acid to prostaglandins. There are two isoforms of COX (Fig. 2), designated as COX-1 and COX-2 (Crofford, 1997; O'Neill and Hutchinson, 1993; Vane *et al.*, 1998; Williams and DuBois, 1996; Wu, 1996). COX-1 as a housekeeping enzyme is

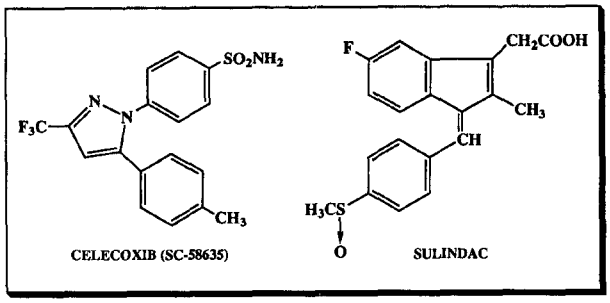

Fig. 1. Chemical structures of representative COX-2 inhibitors that have chemopreventive activities. Celecoxib is the selective inhibitor of COX-2 whereas sulindac has nonselectivity.

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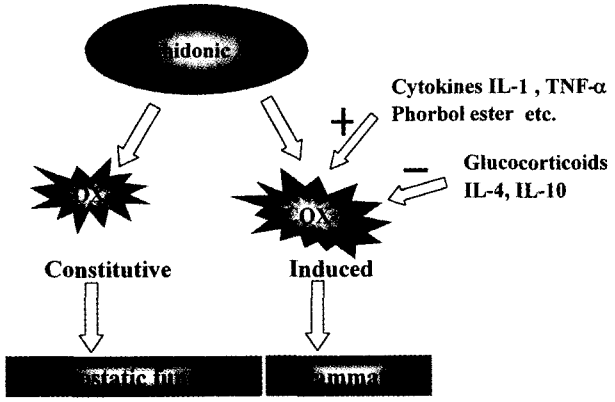

Fig. 2. Schematic representation of roles of COX-1 and COX-2 in arachidonic cascades.

constitutively expressed in tissues throughout the body and has important physiological functions, including cytoprotection of the gastric mucosa, regulation of renal blood flow, and control of platelet aggregation. In contrast, COX-2 is barely detectable under normal physiological conditions, but can be induced by such external stimuli as proinflammatory cytokines, endotoxins, growth factors, viruses, oncogenes, ultraviolet, reactive oxygen species (ROS) and phorbol ester.

II. UP-REGULATION OF COX-2: IMPLICATIONS FOR PATHOGENESIS OF CANCER

Multiple lines of evidence support the notion that COX-2 plays a role in the development of tumors. Thus, inappropriate up-regulation of COX-2 prolongs the survival of malignant or transformed cells. Rat intestinal epithelial cells genetically transformed to overexpress COX-2 exhibit increased adhesion to extracellular matrix proteins and resistance to butyrate-induced apoptosis, which was reversed by sulindac sulfide (Tsujii and DuBois, 1995). Overexpression of COX-2 was also associated with elevated expression of anti-apoptotic Bcl-2 (Tsujii and DuBois, 1995). Conversely, selective COX-2 inhibitors induced apoptosis in certain types of cancer cells (Chan *et al.*, 1998; Grossman *et al.*, 2000; Sheng *et al.*, 1997). Additional evidence that links COX-2 and tumorigenesis includes observations that inhibition of the COX-2 gene in the $Apc^{\Delta 716}$ knockout mouse, a murine model for human familial adenomatous polyposis, suppressed intestinal polyposis (Oshima *et al.*, 1996). Based on these findings, it is conceivable that targeted inhibition of abnormal up-regulation of COX-2 provides one of the most broadly effective and promising approaches to cancer chemoprevention (Subbaramaiah *et al.*, 1997). In animal models of familial adenomatous polyposis, COX-2 inhibitors appeared to be more effective than the traditional NSAIDs in suppressing polyp formation (Taketo, 1998). Celecoxib (SC-58635; structure shown in Fig. 1), a COX-2 selective inhibitor that has been initially manufactured by G.D. Searle & Co. and is being marketed jointly by Searl and Pfizer, has been reported to prevent experimentally induced carcinogenesis (Fischer *et al.*, 1999; Harris *et al.*, 2000; Kawamori *et al.*, 1998; Reddy *et al.*, 2000). A recent clinical trial with celecoxib (the generic name of celebrex) revealed that intake of this COX-2 selective drug at 100 mg and 400 mg doses twice a day for 6 months reduced the mean number of precancerous polyps by 11.9% and 28%, respectively (Chernin, 2000; Steinbach *et al.*, 2000). The additional clinical trials or epidemiologic studies that can assess the chemopreventive potential of celecoxib or related COX-2 selective drugs merit further investigation. Whether the chemopreventive activity of celecoxib and other NSAIDs against colorectal cancer is associated with their inhibition of COX and subsequent PG synthesis is not entirely clear. Recent studies suggest that the induction of programmed cell death (apoptosis) is an important component underlying the action of diverse chemopreventive agents including sulindac and other NSAIDs, which is not necessarily related to their COX inhibitory effects (Elder *et al.*, 1997; Piazza *et al.*, 1997). Furthermore, in COX-null embryofibroblasts, the antiproliferative and anti-neoplastic actions of some NSAIDs were found to be mediated independent of COX expression (Zhang *et al.*, 1999). Some COX-2 inhibitors, such as SC-58125 and NS398), have been shown to sensitize colon and prostate cancer cells, respectively, to apoptosis by down-regulating Bcl-2 (Erickson *et al.*, 1999; Sheng *et al.*, 1998). Very recently, however, Hsu and colleagues (2000) have reported that celecoxib induces apoptosis in androgen responsive (LNCaP) and androgen non-responsive (PC-3) human prostate cancer cells, by blocking the activation of anti-apoptotic kinase Akt, independently of Bcl-2.

III. INTRACELLULAR SIGNALING CASCADES REGULATING COX-2 EXPRESSION

1. Regulation of COX-2 expression by NF-κB

One nuclear target of the intracellular signaling pathways responsible for induction of COX-2 expression is the eukaryotic transcription factor NF-κB. The functionally active NF-κB exists mainly as a heterodimer consisting of subunits of Rel family (e.g., Rel A or p65, p50, p52, c-Rel, v-Rel, and Rel B), which is normally present in the cytoplasm as an inactive complex with the inhibitory protein, IκB. When cells are exposed to such external stimuli as mitogens, proinflammatory cytokines (e.g., TNF-α), ultraviolet, ionizing radiation, viral proteins, bacterial lipopolysaccharides (LPS) and ROS, IκB is rapidly phosphorylated by a specific type of IκB kinase (IKK) with subsequent degradation by proteosomes (Sen and Packer, 1996). Dissociation of IκB from NF-κB releases free NF-κB dimer that eventually translocates to the nucleus, where it induces through binding the *cis*-acting κB element the transcription of *cox*-2 and a large variety of other genes that normally encode cytokines, cell adhesion molecules, growth factors, etc. (Fig. 3).

The 5'-promoter region of COX-2 contains two putative NF-κB binding sites (Fig. 4). Thus, NF-κB has been shown to be a positive regulator of COX-2 expression in diverse cell types. In J774 macrophages stimulated with LPS, production of PGE_2 and 6-keto-$PGF_{1\alpha}$ was significantly reduced by the antioxidant pyrrolidine

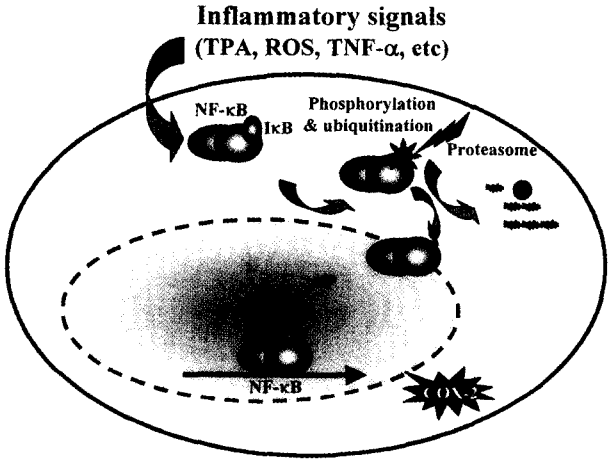

Fig. 3. Regulation of COX-2 expression by NF-κB.

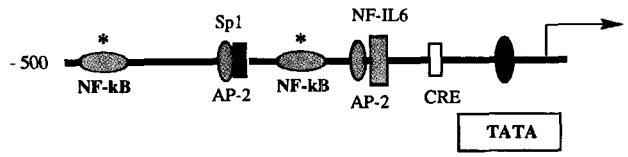

Fig. 4. The COX-2 promoter with transcription factor binding sites. Asterisks indicate the NF-κB binding sites.

dithiocarbamate (PDTC) and the serine protease inhibitor *N*-α-*p*-tosyl-L-lysine chloromethylketone (TPCK) which are inhibitors of NF-κB activation (D'Acquisto *et al.*, 1997). Suppression of the prostanoid production by PDTC or TPCK did not appear to be mediated through direct inhibition of COX-2 activity since these NF-κB inhibitors did not influence the catalytic activity of the enzyme when added to the new media after LPS challenge. According to our study, topical pretreatment of PDTC resulted in dose-related suppression of phorbol ester-induced activation of NF-κB and also caused reduction in the COX-2 level in mouse skin (Y.-J. Surh *et al.*, 2001). Transcriptional up-regulation of COX-2 was also observed in the TNF-α-stimulated mouse osteoblastic cell line (Yamamoto *et al.*, 1995) and also in the IL-1β-treated human pulmonary type II A549 cell line (Newton *et al.*, 1997). A recent study by Schmedtje and coworkers (1997) has demonstrated that hypoxia activated NF-κB in cultured human vascular endothelial cells (HUVEC) concomitantly with increased COX-2 expression. While wild type NF-κB decoy prevented hypoxia induction of COX-2, presumably by binding with the cytoplasmic p65, the mutated or scrambled oligonucleotides failed to block COX-2 expression (Schmedtje *et al.*, 1997).

A colonic epithelial cell line infected with an adenoviral vector containing an NF-κB super-repressor exhibited marked suppression of TNF-α-induced COX-2 expression, compared with those cells uninfected or control cells transfected with the vector alone (Jobin *et al.*, 1998). Acetylsalicylic acid (aspirin) was found to suppress the NF-κB activation through stabilization of IκB (Kopp and Ghosh, 1994). The compound also inhibits TNF-α gene expression in murine macrophages, presumably through down-regulation of NF-κB (Shackelford *et al.*, 1997). Aspirin is known to inhibit the catalytic activity of COX through acetylation of an essential serine residue at the active site of the enzyme. However, its deacetylated product salicylic acid, despite lack of the acetyl group, still inhibits COX-2 activity in-

dependently of NF-κB activation (Mitchell *et al.*, 1997). The transcriptional activity of NF-κB is regulated via an elaborate series of intracellular signal transduction events in response to external stimuli (*vide infra*).

IV. INVOLVEMENT OF MITOGEN-ACTIVATED PROTEIN (MAP) KINASE PATHWAYS IN COX-2 INDUCTION

One of the most extensively investigated intracellular signaling cascades involved in pro-inflammatory responses is the MAP kinase pathway. Of the major MAP kinase subfamily members, extracellular-regulated protein kinase (ERK), c-Jun NH_2-protein kinase (JNK)/stress-activated protein kinase (SAPK) and p38 MAP kinase are most well characterized (Chan-Hui and Weaver, 1998; Davis, 1993; Herlaar and Brown, 1999; Ichijo, 1999; Su and Karin, 1996). These serine/threonine protein kinases are activated through dual phosphorylation at tyrosine and threonine by an upstream MAP kinase-kinase (MKK) in response to a wide array of extracellular stimuli. The activated form of each of the above MAP kinases in turn phosphorylates and activates other kinases or transcription factors, thereby altering the expression of target genes. Blockade of ERK1/2 and p38 MAP kinase activities by PD98059 and SB203580, respectively resulted in partial suppression of LPS-induced expression of COX-2 in RAW 264.7 cells (Hwang *et al.*, 1997). LPS induced the expression of COX-2 protein and its mRNA transcript as well as phosphorylation and activation of ERK2 and p38 MAP kinase in human monocytes (Niiro *et al.*, 1998). The induction of COX-2 and resulting production of PGE_2 were abolished by the specific inhibitors of MAP kinases (Niiro *et al.*, 1998). Monocytes treated with LPS in the presence of the ultrapotent MEK inhibitor U0126 failed to release cytokines and PGE_2 (Scherle *et al.*, 1998). Topically applied U0126 not only prevented TPA-induced phosphorylation of ERK, but also attenuated inflammation in mouse ear (Jaffee *et al.*, 2000). Overexpression of the dominant negative mutant form of JNK resulted in reduced COX-2 expression and PGE_2 production in IL-1β-stimulated rat renal mesangial cells (Guan *et al.*, 1998).

MAP kinases, upstream of NF-κB, regulates activation of this transcription factor by multiple mechanisms. Accumulating evidence indicates that NF-κB activation is modulated by MAP kinase/ERK kinase kinase-1 (MEKK1), an upstream kinase of JNK (Lee *et al.*, 1997) as well as p38 MAP kinase (Schwenger *et al.*, 1998). MEKK1 induced site-specific phosphorylation of IκBα at Ser 32 and Ser 36 in HeLa cells and also directly activated the IκB kinase (IKK) complex (Lee *et al.*, 1997). MEKK1 has been shown to preferentially phosphorylate and thereby activates IKKβ whereas the kinase activity of IKKα is apparently stimulated by NF-κB-inducing kinase (NIK) (Nakano *et al.*, 1998). The resulting phosphorylation of serine residues of IκB targets this inhibitory protein for degradation by the ubiquitin-proteosome pathway, resulting in the re-

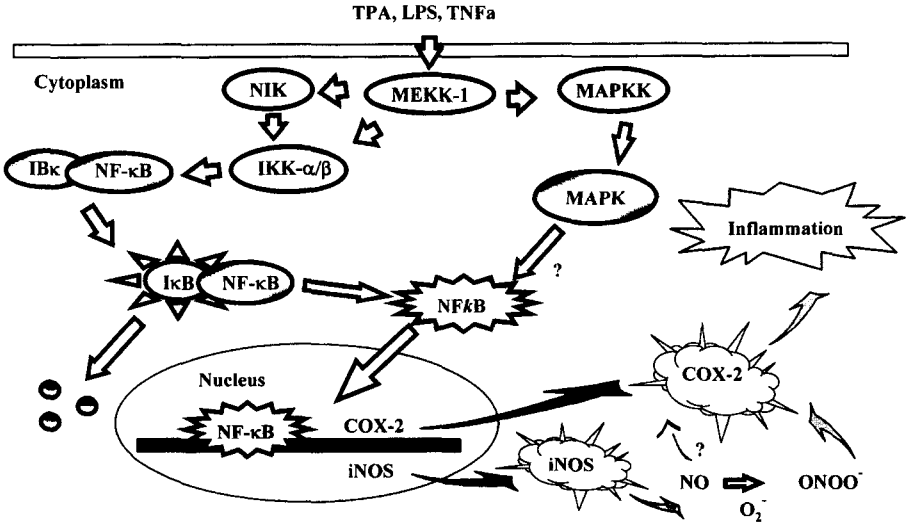

Fig. 5. Intracellular signaling pathways involved in NF-κB activation and subsequent COX-2 induction.

lease of the active NF-κB dimer that translocates to nucleus (Fig. 5). Both IKK and JNK pathways appear to be interconnected since inactive IKKβ blocked vanadate-induced degradation of IκBα and failed to influence the JNK activation by vanadate and blockade of JNK activation resulted in attenuation of vanadate-induced IκBα degradation (Chen *et al.*, 1999).

Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammtory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. An example is curcumin, a yellow pigment derived from turmeric (*Curcuma longa* L., Zingiberaceae) that strongly suppresses tumor promotion. Recent studies from this laboratory have demonstrated that some naturally occurring diarylheptanoids have substantial anti-tumor promotional activities (reviewed by Surh, 1999). Thus, yakuchinone A [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanone] and yakuchinone B [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenylhept-1-en-3-one] present in *Alpinia oxyphylla* Miquel (Zingiberaceae) as well as curcumin attenuate phorbol ester-induced inflammation and skin tumor promotion in mice (Chun *et al.*, 1999a,b). These diarylheptanoids suppressed phorbol ester-induced activation of ornithine decarboxylase and its mRNA expression in mouse skin (Chun *et al.*, 1999a, b). Phorbol ester-induced expression of COX-2 and iNOS was similarly repressed by curcumin and yakuchinones, which appears to be mediated through inactivation of NF-κB (Kim *et al.*, 2000). Beside NF-κB, activator protein 1 (AP-1) was also inactivated by curcumin *in vivo* (Surh *et al.*, 2000) and *in vitro* (Chun *et al.*, 1999a). Capsaicin, a major pungent ingredient of red pepper, also attenuated phorbol ester-stimulated activation of NF-κB and AP-1 in mouse skin (Surh *et al.*, 2000). One of the plausible mechanisms underlying inhibition of NF-κB by aforementioned phytochemicals involves repression of degradation of the inhibitory unit IκB, which hampers subsequent nuclear translocation of p65, the functionally active subunit of NF-κB (Surh *et al.*, 2001).

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