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Signal Transduction Network Leading to COX-2 Induction: A Road Map in Search of Cancer Chemopreventives

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Cancer is still a major global health concern even after an everlasting strive in conquering this dread disease. Emphasis is now given to chemoprevention to reduce the risk of cancer and also to improve the quality of life among cancer afflicted individuals. Recent progress in molecular biology of cancer has identified key components of the cellular signaling network, whose functional abnormality results in undesired alterations in cellular homeostasis, creating a cellular microenvironment that favors premalignant and malignant transformation. Multiple lines of evidence suggest an elevated expression of cyclooxygenase-2 (COX-2) is causally linked to cancer. In response to oxidative/pro-inflammatory stimuli, turning on unusual signaling arrays mediated through diverse classes of kinases and transcription factors results in aberrant expression of COX-2. Population-based as well as laboratory studies have explored a broad spectrum of chemopreventive agents including selective COX-2 inhibitors and a wide variety of anti-inflammatory phytochemicals, which have been shown to target cellular signaling molecules as underlying mechanisms of chemoprevention. Thus, unraveling signaling pathways regulating aberrant COX-2 expression and targeted blocking of one or more components of those signal cascades may be exploited in searching chemopreventive agents in the future.

Key words: Chemoprevention, Cyclooxygenase-2, MAPKs, PI3K-Akt, NF-κB, AP-1, Signaling network, NSAIDs, Antiinflammatory phytochemicals

INTRODUCTION

Despite substantial progress in chemotherapy and radiotherapy, cancer is still the leading cause of death worldwide. Since complete neoplastic transformation is a chronic process occupying more than decades, there are ample opportunities to intervene in the pathophysiology of cancer, especially at early phases of oncogenesis. Recent progress in the understanding of molecular biology of cancer provides us with an in depth knowledge of improper intracellular signaling networks implicated in carcinogenesis. Besides the conventional approach of using cytotoxic drugs to destroy the tumor mass, a strategy involving inhibition, reversal or delay of carcinogenesis by use of nontoxic chemical substances, namely chemoprevention, is now considered as a rational approach for the management of cancer. Various intracellular signaling cascades may undergo abnormal functioning, in the process of multistage carcinogenesis. Therefore, the intracellular signaling network is now considered as a prime target for chemoprevention (Chun and Surh, 2004; Surh 2003).

The emerging evidence suggesting a cause-and-effect relationship between inflammation and cancer (Balkwill and Mantovani, 2001; Clevers 2004; Greten *et al.*, 2004; Itzkowitz and Yio, 2004) sheds lights on the impact of inappropriate induction of cyclooxygenase-2 (COX-2), a key enzyme in the arachidonic acid cascades, and its products, especially prostaglandins (PGs), on premalignant and malignant conversion of cells. Therefore, silencing the symphony in improper cellular signaling that results in aberrant induction of COX-2 and PGs by interfering with the components of upstream signal transduction pathways might offer unlimited scope for developing new chemopreventive agents.

ROLE OF PGs AND COX-2 IN CARCINOGENESIS

There has been increasing evidence from both epide-

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miologic and experimental studies, supporting that pro-inflammatory PGs, including PGE_2 and $PGF_{2\alpha}$, play roles in carcinogenesis as well as inflammation. Elevated levels of PGs have been observed in various types of human cancers (Bennett 1986; Rigas *et al.,* 1993; Vanderveen *et al.,* 1986). Verma and colleagues (Verma *et al.,* 1980) have reported that PGs play crucial roles in the induction of ornithine decarboxylase (ODC) activity, which is a hall mark of tumor promotion, and papillomagenesis in mouse skin treated with phorbol 12-myristate 13-acetate (PMA). Moreover, exposure of various cancer cells to exogenous PGE_2 enhanced cellular proliferation (Bortuzzo *et al.,* 1996; Qiao *et al.,* 1995; Tjandrawinata and Hughes-Fulford, 1997).

In support of the role of PGs in multistage carcinogenesis, several population-based 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 to those not taking NSAIDs (Giovannucci *et al.,* 1995; Greenberg *et al.,* 1993; Schreinemachers and Everson, 1994). Furthermore, the NSAID sulindac reduces the size and the number of intestinal adenomas in patients with familial adenomatous polyposis (Giardiello *et al.,* 1995; Nugent *et al.,* 1993). The cancer chemopreventive properties of NSAIDs have been attributed to their inhibition of COX, which catalyzes the rate-limiting step in the conversion of arachidonic acid to PGs. In early 1990s, COX was found to exist as two distinct forms, COX-1 and COX-2. While COX-1 is constitutively expressed as a house-keeping enzyme in nearly all mammalian tissues and maintains cellular homeostasis, COX-2 is barely detectable under normal physiological conditions. In response to various external stimuli such as pro-inflammatory cytokines, bacterial lipopolysaccharide (LPS), UV, reactive oxygen species (ROS) and phorbol ester, COX-2 is transiently elevated in certain tissues (Fig. 1).

Interestingly, inappropriate up-regulation of COX-2 has been implicated in the pathogenesis of various types of malignancies such as colorectal carcinoma, breast cancer, gastrointestinal cancer, pancreatic cancer, and cancers of head and neck origin (Chan *et al.,* 1999; Grubbs *et al.,* 2000; Pentland *et al.,* 1999; van Rees *et al.,* 2002; Wilgus *et al.,* 2003; Yip-Schneider *et al.,* 2000). Possible mechanisms by which COX-2 contributes to carcinogenesis include promotion of cellular proliferation, suppression of apoptosis, enhancement of angiogenesis and invasiveness, etc. (Surh *et al.,* 2001). Either administration of COX-2 selective rofecoxib or the functional inactivation of the COX-2 gene in $Apc^{\Delta 716}$ knockout mice, a murine model of human adenomatous polyposis, reduced both the number and the size of intestinal polyps (Oshima *et al.,* 1996; Oshima *et al.,* 2001) lending support to an association between COX-2 and tumorigenesis. Moreover, COX-2 overexpressing transgenic mice (Muller-Decker *et al.,* 2002) are highly susceptible to spontaneous tumorigenesis, while COX-2 knockout animals (Tiano *et al.,* 2002) are less sensitive to experimentally-induced tumorigenesis. Overexpression of COX-2 has also been associated with an elevated expression of anti-apoptotic Bcl-2 thereby conferring survival advantage in transformed cells (Tsujii and DuBois, 1995). Thus, rat intestinal epithelial cells genetically transformed to overexpress COX-2 exhibited increased adhesion to extracellular matrix proteins and resistance to butyrate-induced apoptosis, which was reversed by sulindac sulfide. 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).

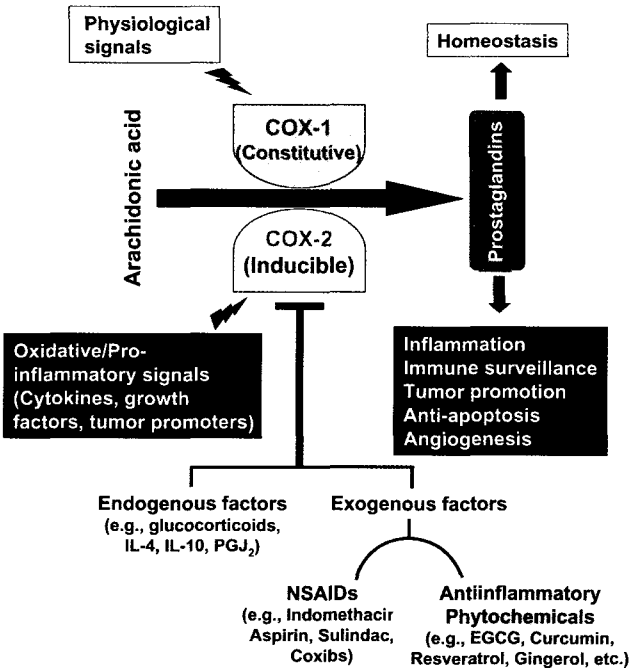

Fig. 1. Differential functions of COX-1 and COX-2

COMPONENTS OF THE CELL SIGNALING NETWORK REGULATING COX-2 EXPRESSION

Although the precise molecular mechanism underlying COX-2 expression is not fully elucidated, distinct roles of aberrant activation of cellular signaling mediated via proline-directed serine-threonine kinases such as mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), phosphatidyl-ionositol-3-kinases (PI3K), Akt/PKB and the down-stream transcription factors in the induction of COX-2 have been documented (Surh, 2003) (Fig. 2). While COX-2 is induced upon exposure of cells or tissues

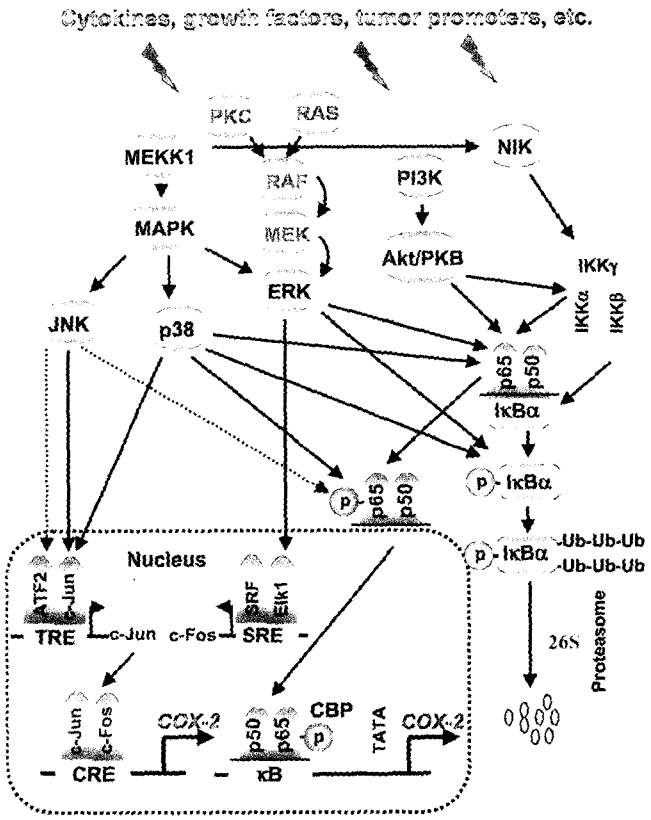

Fig. 2. Intracellular signaling network involved in the regulation of COX-2

to various stimuli including pro-inflammatory cytokines, growth factors, oncogenes and tumor promoters, its expression may also be negatively regulated by factors like glucocorticoids, interleukin-4, interleukin-13, and the anti-inflammatory cytokine interleukin-10 (Surh *et al.*, 2004). Stimuli that induce COX-2 expression switch on improper signaling via PI3K/Akt and Ras/MEK/MAPK pathways, which converge on transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), CCAAT/enhancer binding protein (CEBPβ), cyclic AMP response element binding protein (CREB), etc. The 5' flanking region of COX-2 gene promoter contains specific binding sites for these transcription factors. Therefore, inappropriate activation of afore-mentioned transcription factors, either alone or in combination, results in elevated COX-2 expression. Of the components of cellular signaling, oncogenic *ras* upregulates COX-2 expression in several types of cells by increasing the stability of COX-2 mRNA transcript, and overexpression of COX-2 is associated with tumorigenicity of *ras*-transformed cells (Sheng *et al.*, 2000; Sheng *et al.*, 1998). On the other hand, certain tumor suppressor genes such as p53 negatively regulate COX-2 (Leung *et al.*, 2001; Subbaramaiah *et al.*, 1999). Therefore, involvement of COX-2 in malignant transformation can be partly explained by its association with several known oncogenes and/or tumor suppressor genes. Depending on the stimulus and the cell type, distinct sets of transcription factors and upstream signaling enzymes can differentially modulate the transcription of *COX-2*.

Upstream signaling kinases

MAPK signaling pathway

The MAPK signaling pathway consists of three distinct groups of well characterized serine-threonine protein kinases that include extracellular signal-regulated protein kinases 1/2 (ERK1/2), c-Jun NH_2-terminal kinase (JNK)/ stress-activated protein kinase and p38 MAPK. Cellular pro-inflammatory responses are mediated via activation of one or more members of MAPK family proteins. The activated form of each of the above MAPKs amplifies the signal cascades through phosphorylation of down-stream molecules that include IκB, activating transcription factor-2 (ATF-2), Elk, mitogen and stress-activated kinase (MSK) and transcription factors, thereby altering the expression of target genes including *COX-2* (Chun and Surh, 2004; Deak *et al.*, 1998). Caivano and Cohen (2000), have demonstrated that MAPK cascades are rate limiting for the LPS-induced activation of CREB/ATF1 and transcription of *COX-2* in Raw 264.7 cells. Treatment of human pulmonary epithelial cells with PMA induces COX-2 expression through activation of the Ras/Raf-1/ERK pathway, but not *via* p38 MAPK (Chang *et al.*, 2005). Similarly, activation of the Ras/Raf/MAPKK/ERK pathway is required for COX-2 induction in fibroblasts (Sheng *et al.*, 1998). Recent studies from our laboratory have demonstrated that PMA-induced COX-2 expression in mouse skin is mediated *via* activation of either ERK or p38 MAPK kinase (Chun *et al.*, 2003; Chun *et al.*, 2004). Moreover, pharmacological inhibition (Molina-Holgado *et al.*, 2000; Wang *et al.*, 1998) or dominant-negative knockout of the corresponding MAPK (Guan *et al.*, 1998) has been shown to abrogate the induction of COX-2 or resulting PG production, suggesting the regulatory role of MAPK cascades in COX-2 induction.

PI3K/Akt signaling pathway

Besides MAPK signaling cascades, other upstream kinases can contribute to aberrant expression of COX-2. Tang *et al.* (2001) have demonstrated that UVB-induced COX-2 expression in human keratinocytes is mediated via phosphorylation of Akt at both threonine-308 and serine-473 residues leading to inactivation of its down-stream kinase glycogen synthase kinase-3β. Either pharmacological inhibition of Akt or overexpression of a dominant-negative Akt mutant or wild-type GSK-3β abrogated UVB-mediated induction of COX-2. Moreover, inhibition of GSK-3β by lithium resulted in the elevated protein expression and promoter activity of COX-2. Compared to the non-

tumorigenic keratinocytes (HaCaT), human skin cancer (HSC-5) cells were found to exhibit not only elevated levels of PI3K activity but also COX-2 protein expression, which was diminished by treatment with PI3K inhibitor LY294002, suggesting the PI3K-mediated regulation of COX-2 expression (Takeda *et al.,* 2004). In contrast, IL-1β-induced expression of COX-2 protein and its mRNA transcript in human colon cancer (HT-29) cells has been shown to be mediated via the MAPK-NF-κB signaling pathway, but not by the PI3K-mediated pathway (Liu *et al.,* 2003). The induction of COX-2 in rat intestinal epithelial (IEC-6) cells transfected with an inducible K-RasVal12 cDNA was mediated via activation of both ERK and Akt/PKB. According to this study, pharmacological inhibition of either ERK or PI3K/Akt or expression of dominant negative Akt partially blocked the induction of COX-2 by K-Ras (Sheng *et al.,* 2001). A regulatory role of PI3K/Akt signaling in aberrant expression of COX-2 and PGE_2 synthesis in endometrial cancer cells has recently been reported (St-Germain *et al.,* 2004a). In this study, endometrial cancer cells (RL 95-2 and Ishikawa) with mutated PTEN, a negative regulator of Akt, exhibited elevated levels of COX-2 protein as well as mRNA as compared to cells expressing wild type PTEN (HEC 1-A). Pharmacological inhibition or transfection with dominant negative Akt vector diminished COX-2 expression as well as Akt phosphorylation in PTEN-mutated cells. In another study, treatment of PTEN-mutated RL 95-2 and Ishikawa cells with wortmanin and LY294002, specific inhibitors of PI3K, down-regulated IκBα phosphorylation, reduced NF-κB nuclear activity and decreased COX-2 expression, suggesting that PI3K/Akt regulates COX-2 expression in endometrial cancers *via* activation of NF-κB-mediated signaling (St-Germain *et al.,* 2004b).

Signaling through other kinases

In addition to afore-mentioned serine-threonine kinases, aberrant activation of cytosolic tyrosine kinases, PKC and IκB kinases (IKKs) also contribute to COX-2 overexpression (Huang *et al.,* 2003). Treatment of human lung epithelial (NCI-H292) cells with TNF-α activated phospholipase C-γ2 *via* an upstream tyrosine kinase and subsequently PKC-α and protein tyrosine kinase, which in turn, activated NF-κB *via* NF-κB-inducing kinase (NIK) and IKK1/2, and resulted in induction of COX-2 expression and PGE_2 release (Chen *et al.,* 2000). Simultaneous treatment of human gingivial fibroblast cells with extracellular growth factor (EGF) and interleukin-1β (IL-1β) resulted in enhanced COX-2 mRNA levels and synergistic stimulation of PGE_2 biosynthesis, which was diminished by tyrosine kinase inhibitors, herbimycin A and PD 153035 hydrochloride (Yucel-Lindberg *et al.,* 1999). IL-1β-induced COX-2 expression and PGE_2 production in human pulmonary epithelial (A549) cells were inhibited by tyrosine kinase inhibitors tyrphostin or erbstatin (Akarasereenont and Thiemermann, 1996). Genistein with tyrosine kinase inhibitory activity diminished LPS-induced COX-2 expression and PGF2α production in J774.2 macrophages (Akarasereenont *et al.,* 1994). Activated PKC and Ras signaling pathways have been shown to regulate *COX-2* expression at the transcriptional level (Marks *et al.,* 2000). Wang *et al.* (2001) demonstrated that the induction of epidermal COX-2 expression and PGE_2 synthesis has been increased significantly in K5-PKCα transgenic mice treated with PMA as compared to wild-type mice. The study also revealed that PKC inhibitors GF-109203X and H7 blocked COX-2 induction by PMA. Subbaramaiah and coworkers (2002) have shown that HER-2/neu stimulates COX-2 transcription *via* the Ras signaling pathway in cultured human mammary epithelial cells.

Transcription factors

The expression of COX-2 is regulated transcriptionally by a variety of transcription factors (Dannenberg *et al.,* 2001; Shao *et al.,* 2000). In general, the promoter region of the COX-2 gene contains a canonical TATA box and various putative transcriptional regulatory elements, such as NF-κB, NF-IL6 (C/EBP), PEA3, NFAT, CRE, AP-2, and SP-1 (Kosaka *et al.,* 1994). Inappropriate activation of these transcription factors can lead to overexpression of COX-2. The role of individual transcription factors in COX-2 induction has recently been reviewed by Chun and Surh (2004). One of the most extensively investigated transcription factors involved in the induction of COX-2 expression is NF-κB, which is an inducible and ubiquitously expressed transcription factor. A growing body of evidence indicates that NF-κB plays a central role in general inflammatory as well as immune responses (Marx, 2004; Pikarsky *et al.,* 2004; Shishodia and Aggarwal, 2004). Recent studies have suggested NF-κB as a potential link between inflammation and cancer. NF-κB has been shown to act as a positive regulator of LPS-induced COX-2 expression in murine macrophages (D'Acquisto *et al.,* 1997) and human colon adenocarcinoma cell lines (Kojima *et al.,* 2000). In the former study, production of PGE_2 and 6-keto-$PGF_{1\alpha}$ in LPS-stimulated J774 macrophages has been reduced by the antioxidant pyrrolidine dithiocarbamate (PDTC) and the serine protease inhibitor *N*-α-*p*-tosyl-L-lysine chloromethylketone, which are inhibitors of NF-κB activation (D'Acquisto *et al.*, 1997). According to Liu *et al.* (1999), pretreatment of rats with PDTC suppressed LPS-induced NF-κB activation and COX-2 expression. Moreover, the expression of COX-2 in IL-1β-stimulated amnion mesenchymal cells was blocked by the NF-κB inhibitor SN50, but not by its inactive form SN50M (Yan *et al.,* 2002).

In resting cells, NF-κB is normally sequestered in the cytoplasm as an inactive complex with the inhibitory protein, IκB. Exposure of cells to mitogens, pro-inflammatory cytokines, UV, ionizing radiation, viral proteins, bacterial toxins etc. initiates signals that, by turning on either IKK or MAPK, leading to rapid phosphorylation and ubiquitination of IκB proteins, which are then degraded by proteasomes. Dissociation of IkB from NF-κB allows the activated free dimer to translocate to the nucleus, where it binds to an NF-κB element located in the promoter or enhancer regions of *COX-2* and other target genes, controlling their expression. However, IκBα degradation-independent nuclear translocation of NF-κB has also been reported (Canty *et al.,* 1999; Wong *et al.,* 2003). Certain stimuli such as H_2O_2 or hypoxia followed by reoxygenation may cause phosphorylation of IκBα at tyrosine residues rendering this protein resistant to proteasomal degradation but rather facilitating its dissociation from NF-κB (Imbert *et al.,* 1996; Koong *et al.,* 1994; Takada *et al.,* 2003).

NF-κB DNA binding activity is not necessarily associated with its transcriptional activity (Harnish *et al.,* 2000; Takahashi *et al.,* 2002). Inhibitors of some upstream kinases, such as PI3K/Akt, p38 MAPK and protein kinase A (PKA), may block the transcriptional activity of NF-κB without affecting its nuclear translocation (Madrid *et al.,* 2001; Sizemore *et al.,* 1999; Wesselborg *et al.,* 1997; Zhong *et al.,* 1998). The efficient transcriptional activation of NF-κB depends on the phosphorylation of its active subunit p65/RelA (Ghosh and Karin, 2002). It has been reported that TNF-α and IL-1 stimulate p65/RelA phosphorylation and subsequent NF-κB transactivation *via* mechanisms distinct from those that involve the IκBα phosphorylation and subsequent nuclear translocation of NF-κB (Baldwin, 1996; Bird *et al.,* 1997; Sizemore *et al.,* 1999; Wang and Baldwin, 1998). The upstream IKK signalsome has been shown to mediate phosphorylation of both IkB and p65 (Sakurai *et al.,* 1999; Yang *et al.,* 2003).

Since transcriptional co-activators such as cyclic AMP response element binding protein-binding protein (CBP)/p300 can link enhancer-bound transcription factors to general transcription machinery (Zhong *et al.,* 1998), the interaction of afore-mentioned transcription factors with CBP/p300 is a critical event in transcriptional regulation of *COX-2*. It has been reported that cotransfection of cells with CBP/p300 enhances NF-κB-dependent transcription (Gerritsen *et al.,* 1997). Zhong and colleagues (1998) have demonstrated that the association of NF-κB with CBP/p300 occurs either by a phosphorylation-independent mechanism or through PKA-dependent phosphorylation of p65/RelA.

The up-regulation of *COX-2* is also mediated by AP-1, another important eukaryotic transcription factor consisting of basic leucine zipper proteins from Jun and Fos family. Like NF-κB, activation of this transcription factor is mediated by MAPKs in response to various stimuli (Chun and Surh, 2004). In v-*src*-transformed cells, COX-2 expression requires the AP-1 proteins, *c*-Jun and *c*-Fos, that activate COX-2 transcription through binding to the CRE site present at the 3'-end of the *COX-2* promoter (Xie and Herschman, 1995). C/EBP transcription factors are also involved in regulating *COX-2* transcription. COX-2 induction by LPS was profoundly defective in C/EBPβ (-/-) macrophages, essentially due to impaired transcriptional induction *via* the C/EBP promoter element. In contrast, C/EBP was totally dispensable for *COX*-2 transcriptional induction in fibroblasts in response to PMA treatment or v-*src* transfection, suggesting a cell specific nature of COX-2 regulation (Gorgoni *et al.,* 2001). However, binding of only one transcription factor within a promoter region sometimes is not sufficient to induce gene expression in general. Several studies have demonstrated that expression of COX-2 requires simultaneous activation of a combination of transcription factors (Inoue *et al.,* 1995; Miller *et al.,* 1998). According to a recent study by Allport and colleagues (2000), NF-κB and AP-1 are required for COX-2 expression in the amnion epithelial cell line.

Modulation of chromatin structures by DNA methylation or histone acetylation is known to affect transcription of certain genes (Bird and Wolffe, 1999). Methylation status of the 5' flanking region of *COX-2* is closely associated with nullified expression of COX-2 (Kikuchi *et al.,* 2002). Because treatment of the methyltransferase inhibitor 5-deoxy-2-azacytidine readily induced expression of COX-2, aberrant methylation of the COX-2 promoter could suppress the expression of the enzyme even in the presence of the transcription factor. This study also demonstrated that despite restoration of histone acetylation by the histone deacetylase inhibitor trichostatin A in a methylated cell line, COX-2 expression was not recovered unless methylation was inhibited (Kikuchi *et al.,* 2002). However, combined treatment of a methylation inhibitor and a histone deacetylase inhibitor reactivated gene transcription synergistically, indicating that histone deacetylation does play a role in part in methylation-dependent gene repression.

CELLULAR SIGNALING MOLECULES REGULATING COX-2 EXPRESSION AS TARGETS FOR CANCER CHEMOPREVENTION

In the context of emerging evidence of a causal relationship between inflammation and cancer, exploitation of anti-inflammatory substances as chemopreventives might fortifying the arsenal in fighting cancer. Since persistent overexpression of COX-2 is associated with malignant

transformation, targeted inhibition of this pro-inflammatory enzyme by anti-inflammatory substances would be a promising strategy for chemoprevention. Chemopreventive potential of NSAIDs and some anti-inflammatory phytochemicals has been partly attributed to the inhibition of aberrant expression of COX-2 in inflammation-associated carcinogenesis. Since COX-2 is up-regulated via orchestrated cellular signaling network, which is usually turned on by various noxious stimuli, components of signaling pathways, including protein kinases and transcription factors, are potential targets for developing chemopreventive agents.

NSAIDs with COX-2 inhibitory activity

In general, the undesired side effects of NSAIDs have been ascribed to disruption of COX-1-derived synthesis of prostanoids that are involved in maintaining homeostasis, while inhibition of COX-2-dependent PG synthesis accounts for the anti-inflammatory, analgesic, and antipyretic effects of these drugs. Thus, the development of NSAIDs with selective COX-2 inhibitory property has been attempted to avoid unwanted side effects or toxicity resulting from COX-1 inhibition by conventional NSAIDs (Fig. 3). Celecoxib, the first selective COX-2-inhibitors being initially approved for the treatment of adult rheumatoid arthritis and osteoarthritis, has recently been recognized as a promising

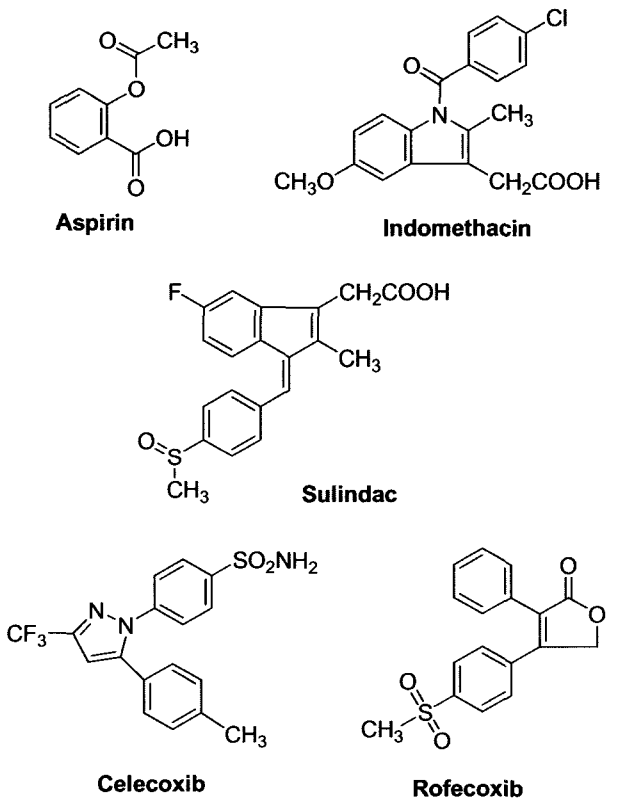

Fig. 3. Chemical structures of selected NSAIDs

chemopreventive agent. Celecoxib was approved in the USA as an adjunct to standard care for patients with familial adenomatous polyposis based on the results of a randomized, double-blind, placebo-controlled trial with 77 patients (Steinbach *et al.*, 2000). Celecoxib has also been shown to inhibit experimentally-induced tumorigenesis in several animal models (Alshafie *et al.*, 2000; Grubbs *et al.*, 2000; Harris *et al.*, 2000; Kawamori *et al.*, 1998). Dietary administration of celecoxib exhibited a significant chemopreventive activity against UV-induced skin carcinogenesis in hairless SKH-1 mice (Fischer *et al.*, 1999). Pentland *et al.* (1999) demonstrated that orally administered celecoxib blocks the promotion stage of photocarcinogenesis in hairless mice.

Molecular mechanisms of chemoprevention with celecoxib, especially targeting signal transduction pathways leading to aberrant COX-2 expression, have been reviewed recently by Chun and Surh (2004). Study from our laboratory demonstrated that topical application of celecoxib prevented mouse skin tumor formation and inhibited the protein expression and enzyme activity of ODC (K.-S. Chun and Y.-J. Surh, manuscript in preparation). We also reported that celecoxib inhibited PMA-induced COX-2 protein and mRNA expression in mouse skin by blocking p38 MAPK-mediated AP-1 activation (Chun *et al.*, 2004). Celecoxib suppressed NF-κB activation induced by various stimuli, including TNF-α, phorbol ester, okadaic acid, LPS, and IL-1β (Shishodia *et al.*, 2004). The inhibition of TNF-α-induced activation of NF-κB by celecoxib was mediated *via* suppression of IKK activation, leading to abrogation of IκBα phosphorylation and degradation and subsequent attenuation of phosphorylation and nuclear translocation of p65. This study also demonstrated that the activation of Akt, which is required for TNF-α-induced NF-κB activation, as well as the interaction of Akt with IKK was also suppressed by celecoxib. In addition, celecoxib inhibited TNF-α-induced activation of JNK, p38 MAPK and ERK as well as COX-2 promoter activity (Shishodia *et al.*, 2004). Inhibition of COX-2 by celecoxib delayed tumor growth and metastasis in xenograft tumor models and suppressed basic fibroblast growth factor-2-induced neovascularization of the rodent cornea (Leahy *et al.*, 2002). Zweifel *et al.* (2002) reported that inhibition of COX-2 by celecoxib resulted in loss of intratumor PGE_2 levels and reduced the growth of 1483 human head and neck xenograft tumors in a dose-dependent manner. Niederberger *et al.* (2001) recently showed that high concentrations of celecoxib did not inhibit, rather activated NF-κB and induced NF-κB-dependent gene transcription. Similarly, administration of a low dose of indomethacin to BALBc mice inoculated subcutaneously with murine colon 26 adenocarcinoma cells decreased the activation of NF-κB and serum levels of TNF-α and IL-

6, but failed to inhibit NF-κB at a relatively high dose (Zhou *et al.*, 2003). Rofecoxib, another COX-2 selective NSAIDs, inhibited LPS-induced COX-2 expression by suppressing NF-κB activation (Callejas *et al.*, 2003). However, rofecoxib inhibited COX-2 activity, but not the protein expression, as revealed by suppression of PGE_2 formation in $Apc^{\Delta 716}$ knockout mice (Evans 2003). Aspirin and salicylate at therapeutic concentrations inhibited COX-2 protein expression through interference with binding of C/EBPβ to its cognate site on the COX-2 promoter (Wu, 2003).

Anti-inflammatory phytochemicals

Numerous anti-inflammatory phytochemicals present in our daily diet as natures bounty have been shown to possess strong cancer chemopreventive potential in both epidemiological and experimental studies. Examples of extensively investigated phytochemicals are curcumin from turmeric, resveratrol from red wine and grapes, capsaicin from hot chilli pepper, epigallocatechin gallate (EGCG) from green tea, caffeic acid phenethyl ester from honey bee propolis, gingerol from ginger, etc. (reviewed by Surh, 2003). Fig. 4 illustrates chemical structures of some representative anti-inflammatory phytochemicals that have been reported to posses chemopreventive properties.

Curcumin

Curcumin (diferuloylmethane), a yellow pigment present in the rhizome of turmeric (*Curcuma longa* L., Zingiberaceae), has been reported to inhibit PMA-induced hyperplasia, activity and expression of ODC, ROS generation and oxidative DNA damage as well as papilloma formation in mouse skin (Conney *et al.*, 1997; Huang *et al.*, 1997). Research from our laboratory demonstrated that either topical application in mouse skin or treatment of cultured HL-60 cells with curcumin inhibited activation of AP-1 and NF-κB (Surh *et al.*, 2000). Curcumin has also been shown to inhibit COX-2 expression by blocking activation of both AP-1 and NF-κB in human BV2 microglial cells stimulated with LPS (Kang *et al.*, 2004). Recently, we reported that topical application of curcumin abrogated PMA-induced COX-2 expression in female ICR mouse skin by blocking activation of ERK and NF-κB (Chun *et al.*, 2003). Li and colleagues (2004) also demonstrated that curcumin suppressed constitutive overexpression of COX-2 in pancreatic cancer cells by blocking IKK activity and NF-κB DNA binding. Similarly, treatment of human head and neck squamous cell carcinoma (MDA 686LN) cells (Aggarwal *et al.*, 2004b) and cigarette smoke-treated human lung epithelial cells (Shishodia *et al.*, 2003) with curcumin resulted in the down-regulation of COX-2 expression *via* suppression of IKK-mediated activation of NF-κB, which was constitutively expressed in this cell line.

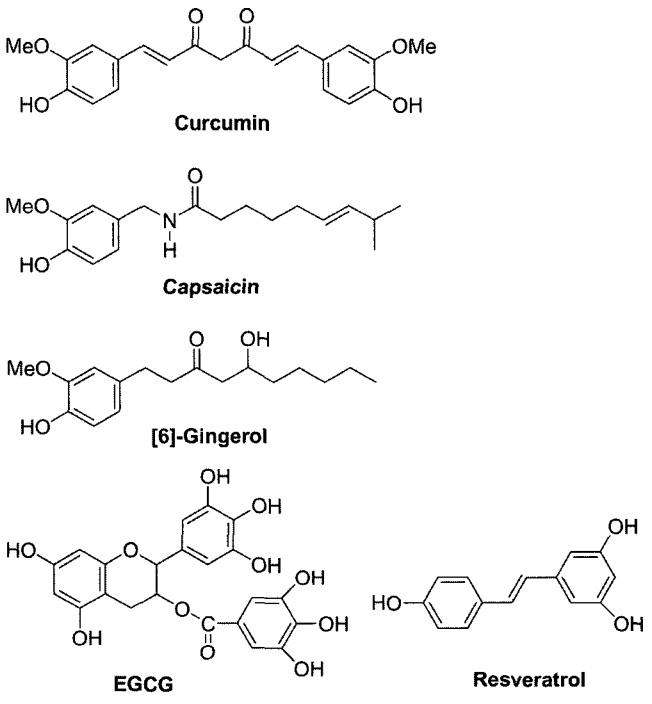

Fig. 4. Selected chemopreventive phytochemicals with anti-inflammatory properties

EGCG

An inverse correlation between green tea consumption and risk of cancer has been noted in several epidemiological studies (Park and Surh, 2004). One of the major constituents of green tea is EGCG, which is known to possess anti-oxidant, anti-inflammatory and chemopreventive properties. One of the molecular mechanisms underlying chemopreventive activity of EGCG is the suppression of COX-2. A recent study from our laboratory has revealed that intragastric administration of EGCG inhibited PMA-induced COX-2 expression in female ICR mouse skin and human breast epithelial (MCF-10A) cells (Kundu *et al.*, 2003). According to this study EGCG abrogated activation of ERK and p38 MAPK in PMA-treated mouse skin. Intraperitoneal administration of EGCG inhibited both COX-2 expression and PGE_2 production in *N*-nitrosomethylbenzylamine-induced rat esophageal cancer (Li *et al.*, 2002). Similarly, the suppression of both protein and mRNA expression of COX-2 in human prostate cancer cells by EGCG was reported (Hussain *et al.*, 2005). Moreover, EGCG down-regulated 2,2'-azobis-(2-amidinopropane)-dihydrochloride-induced COX-2 expression in HaCaT cells by blocking p38 MAPK (Cui *et al.*, 2004). While EGCG was shown to diminish aberrant COX-2 expression in cells/tissues exposed to various stimuli, the compound also induced COX-2 expression and PGE_2 formation in Raw 264.7 cells (Park *et al.*, 2001).

Resveratrol

Resveratrol, a polyphenol present in red wine, grapes and other edible plant species, has anti-oxidant, immunomodulatory, anti-inflammatory and chemopreventive properties. Molecular mechanisms underlying chemopreventive activity of resveratrol have recently been reviewed (Aggarwal *et al.,* 2004a; Kundu and Surh, 2004). The anti-inflammatory and antitumor promoting potential of resveratrol was reported in a pioneering study by John M. Pezzuto and colleagues who demonstrated that the compound inhibited COX-2 activity and mouse skin tumor formation (Jang *et al.,* 1997). Recent studies demonstrated that resveratrol significantly inhibited COX-2 protein expression in trinitrobenzenesulfonic acid-induced rat colonic inflammation (Martin *et al.,* 2004) and LPS-, PMA- or H_2O_2 treated mouse peritoneal macrophages (Martinez and Moreno, 2000; Murakami *et al.,* 2003). We also reported that resveratrol inhibited PMA-induced COX-2 expression by blocking activation of ERK and p38 MAPK as well as AP-1 DNA binding in female ICR mouse skin *in vivo* (Kundu *et al.,* 2004). In addition, resveratrol was found to inhibit PMA-induced COX-2 expression in mouse skin by suppressing activation of IKK-NF-κB mediated signaling (J.K. Kundu and Y.-J. Surh, unpublished data). Similarly, topical application of resveratrol inhibited COX-2 protein expression in SKH-1 hairless mouse skin stimulated with UVB (Aziz *et al.,* 2004). According to Banerjee *et al.* (2002), the inhibition of 7,12-dimethylbenzanthracene-induced rat mammary carcinogenesis by resveratrol was attributed to suppression of NF-κB activation and COX-2 expression. Resveratrol was reported to reduce PMA-induced PGE_2 production by down-regulating *COX-2* gene transcription in human mammary and oral epithelial cells *via* modulation of PKC, ERK and AP-1 activity (Subbaramaiah *et al.*, 1999; Subbaramaiah *et al.,* 1998).

Gingerol

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is another widely used condiment that is a potential source of anti-inflammatory phytochemicals. One of the major pungent principles of ginger is [6]-gingerol, which was found to inhibit epidermal growth factor-induced AP-1 activation and neoplastic transformation in mouse epidermal JB6 cells (Bode *et al.,* 2001). [6]-Gingerol inhibited tumor promotion, PMA-induced ODC activity and TNF-α production in mouse skin *in vivo* (Park *et al.,* 1998). A recent study from this laboratory revealed that topical application of [6]-gingerol inhibited PMA-induced COX-2 expression in mouse skin by suppressing NF-κB activation (Kim *et al.,* 2004). Furthermore, [6]-gingerol inhibited degradation of IκBα and nuclear translocation of p65 *via* a mechanism regulated by p38 MAPK (Kim *et al.,* 2005).

Capsaicin

Recently, attention has been focused on chemopreventive potential of capsaicin, a pungent principle of hot chilli pepper (*Capsicum annuum* L., Solanaceae), which was under dispute to be a carcinogen or co-carcinogen (Surh and Lee, 1995, 1996). Studies from our laboratory demonstrated that the compound applied topically to mouse skin did not show tumor promoting activity, but rather inhibited PMA-induced papilloma formation (Park and Surh, 1997; Park *et al.,* 1998). Moreover, capsaicin inhibited the activation of NF-κB by blocking IκBα degradation and p65 nuclear translocation (Han *et al.,* 2001). A recent study by Duvoix *et al.* (2004) demonstrated that treatment of K562 and U937 leukemia cells with capsaicin significantly inhibited reporter gene expression as well as TNF-α- and PMA-induced DNA binding of AP-1 and NF-κB, which are prime regulators of COX-2 gene expression. Moreover, capsaicin was found to inhibit LPS- and PMA-induced COX-2 expression and PGE_2 production as well as LPS-induced NF-κB and AP-1 activation in Raw 264.7 cells (Chen *et al.,* 2003). Similarly, capsaicin blocked COX-2 enzyme activity by inhibiting IκBα degradation and resultant NF-κB activation in LPS-stimulated peritoneal macrophages (Kim *et al.,* 2003).

Miscellaneous anti-inflammatory phytochemicals

Besides the phytochemicals listed above, a broad spectrum of anti-inflammatory phytochemicals have been shown to regulate COX-2 expression as a mechanism of antitumor promoting effect. Some anti-inflammatory constituents from *Alpinia* species as well as ginseng metabolites may exert antitumor promoting effects by targeting COX-2. Examples are Yakuchinone A and B from *Alpinia oxyphylla*, Rg3 from *Panax ginseng* C.A. Mayer and IH-901 (20-*O*-beta-D-glucopyranosyl-20(S)-protopanaxadiol), a metabolite derived from protopanaxadiol-type ginsenosides formed by intestinal bacteria (Chun *et al.,* 2002a; Chun *et al.,* 2002b; Keum *et al.,* 2003; Lee *et al.,* 2004).

CONCLUDING REMARKS

Numerous studies during the past decade have demonstrated unequivocally that COX-2 is implicated in pathophysiology of human carcinogenesis. Prolonged intake of some conventional NSAIDs, such as aspirin and sulindac, has been shown to reduce the risk of colon cancer and other malignancies. More recently, COX-2 specific inhibitors have received considerable attention because of their promising chemopreventive potential. An example is celecoxib that was approved by the US FDA for the complimentary treatment for patients with familial adenomatous polyposis who are genetically susceptible to colorectal cancer due to hereditary loss of the APC tumor

suppressor gene. Besides NSAIDs, some naturally occurring anti-inflammatory substances, particularly those derived from edible plants, also possess substantial COX-2 inhibitory effects and hence are good candidate chemopreventives. The induction of COX-2 is mediated by a series of upstream signaling molecules including a distinct set of kinases and transcription factors. Due to unexpected side effects recently observed with even the best-selling COX-2 selective inhibitors, it is rationale to look for relatively safe natural COX-2 inhibitors, particularly those of plant origin, as an arsenal for the prevention of cancer. Elucidation of intracellular signaling targets for COX-2 inhibition with such anti-inflammatory phytochemicals is necessary before they are considered to be developed as potential chemopreventive agents.

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