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Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention

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Abstract

Expression of cyclooxygenase-2 (COX-2) has been reported to be elevated in human colorectal adenocarcinoma and other tumors, including those of breast, cervical, prostate, and lung. Genetic knock-out or pharmacological inhibition of COX-2 has been shown to protect against experimentally-induced carcinogenesis. Results from epidemiological and laboratory studies indicate that regular intake of selective COX-2 inhibitors reduces the risk of several forms of human malignancies. Thus, it is conceivable that targeted inhibition of abnormally or improperly elevated COX-2 provides one of the most effective and promising strategies for cancer chemoprevention. The COX-2 promoter contains a TATA box and binding sites for several transcription factors including nuclear factor-κB (NF-κB), nuclear factor for interleukin-6/CCAAT enhancer-binding protein (NF-IL6/C/EBP) and cyclic AMP response element (CRE) binding protein. Upregulation of COX-2 is mediated by a variety of stimuli including tumor promoters, oncogenes, and growth factors. Stimulation of either protein kinase C (PKC) or Ras signaling enhances mitogen-activated protein kinase (MAPK) activity, which, in turn, activates transcription of *cox-2*. Celecoxib, the first US FDA approved selective COX-2 inhibitor, initially developed for the treatment of adult rheumatoid arthritis and osteoarthritis, has been reported to reduce the formation of polyps in patients with familial adenomatous polyposis. This COX-2 specific inhibitor also protects against experimentally-induced carcinogenesis, but the underlying molecular mechanisms are poorly understood. The present review covers the signal transduction pathways responsible for regulating COX-2 expression as novel molecular targets of chemopreventive agents with celecoxib as a specific example.

Keywords: Cyclooxygenase-2; Celecoxib; Transcription factors; Mitogen-activated protein kinase; Chemoprevention

1. Introduction

One of the most promising approaches to reduce the risk of cancer is chemoprevention [1,2]. Chemoprevention is the attempt to use nontoxic natural and synthetic compounds or their mixtures to intervene in the early precancerous stages of carcinogenesis, before invasive characteristics are manifest. Chronic inflammation has been considered to promote tumor development by multiple mechanisms. In line with this notion, NSAIDs have been shown to inhibit malignant transformation in various animal models and cultured cells. Moreover, frequent intake of such drugs seems to be associated with the reduced risk of colorectal cancer and other malignancies in humans, and in clinical studies NSAID treatment has been shown to halt or regress tumor growth in patients bearing polyps [3,4].

COX or prostaglandin H_2 synthase is the key enzyme in the biosynthesis of the PGs mediating inflammation and other important physiological processes. In the early 1990s, COX was demonstrated to exist as two distinct isoforms [5]. COX-1, as a housekeeping enzyme, is constitutively expressed in nearly all tissues, and mediates physiological responses (e.g., cytoprotection of the stomach, platelet aggregation, and regulation of renal blood

Abbreviations: COX, cyclooxygenase; PG, prostaglandin; NSAIDs, nonsteroidal anti-inflammatory drugs; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; MEK1/2, mitogen-activated protein/extracellular signal-regulated kinase kinase; PKC, protein kinase C; NF-κB, nuclear factor-κB; AP-1, activator protein 1; PEA3, polyomavirus enhancer activator 3; CRE, cyclic AMP response element; NF-IL6/C/EBP, interleukin-6/CCAAT enhancer-binding protein; NFAT, nuclear factor of activated T cells; CBP, cyclic AMP response element binding protein (CREB) binding protein; ETS, E twenty-six; PPAR, peroxisome-activated receptor; IKK, IκBα kinase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz[*a*]anthracene; ODC, ornithine decarboxylase; LPS, lipopolysaccharide; SRE, serum-response element; TRE, TPA-response element; IL-1β, interleukin 1β

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flow). On the other hand, COX-2 expressed by cells that mediate inflammation (e.g., macrophages, monocytes, synoviocytes) has been recognized as the isoform that is primarily responsible for the synthesis of the prostanoids involved in pathological processes, particularly those related to acute and chronic inflammatory states.

2. COX-2 and cancer

Multiple lines of compelling evidence support that COX-2 plays a crucial role in carcinogenesis (Fig. 1). Many human malignancies produce more PGs than the normal tissues from which they arise [6–9]. Increased synthesis of PGs in transformed cells and tumors can be a consequence of enhanced expression of COX-2 [10]. PGs are believed to be important in the pathogenesis of cancer because of their effects on cell proliferation, angiogenesis, immune surveillance, and apoptosis [11–14]. The hypothesis that PGs contribute to carcinogenesis is supported by epidemiologic and experimental findings that inhibitors of COX, including NSAIDs, protect against a variety of tumors [15–22]. In addition, clinical studies also revealed that sulindac, a dual inhibitor of COX-1 and COX-2, caused a decrease in the number of colorectal polyps in patients with familial adenomatous polyposis which is a hereditary precancerous disease developed as a result of loss of the APC tumor suppressor gene [23]. The most specific data that support a cause-effect connection between COX-2 and tumorigenesis come from genetic studies. Transgenic mice that are genetically engineered to overexpress human *cox-2* in mammary glands developed focal mammary gland hyperplasia, dysplasia and metastatic tumors [24]. These findings are consistent with the idea that elevated expression of COX-2, under some conditions, can facilitate tumor formation. In a related study, transgenic mice that overexpress *cox-2* in skin developed epidermal hyperplasia and dysplasia [25]. Oshima et al. constructed transgenic mice simultaneously expressing *cox-2* in the gastric epithelial cells. The transgenic mice developed metaplasia, hyperplasia and malignant growths in the glandular stomach with heavy macrophage infiltrations [26]. Consistent with these studies, knocking out *cox-2* markedly reduced the development of intestinal tumors and skin papillomas [27–29]. In contrast, there are opposite findings that *cox-2* transgenics develop tumors at a much lower frequency than do their littermate controls [30].

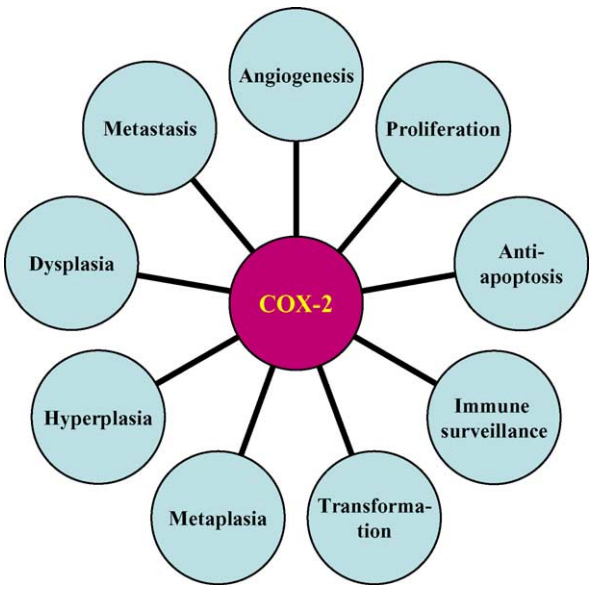

Fig. 1. Schematic representation of roles of COX-2 in carcinogenesis.

3. Transcriptional regulation of COX-2 expression

Overexpression of COX-2 appears to be a consequence of both increased transcription and enhanced mRNA stability [31,32]. Tumor promoters, growth factors, oncogenes and cytokines stimulate *cox-2* transcription via PKC and Ras-mediated signaling [33]. TPA treatment of K5-PKCα transgenic mice resulted in a five-fold increase in epidermal COX-2 expression and a two- to three-fold increase in PGE_2 levels above those observed in TPA-treated wild-type mice. PKC inhibitors GF-109203X or H7 blocked COX-2 induction by TPA [34]. Subbaramaiah et al. [35] showed that HER-2/neu stimulates COX-2 transcription via the Ras signaling pathway in cultured human mammary epithelial cells. The *HER-2/neu* (*erbB-2*) gene encodes a 185 kDa transmembrane receptor with tyrosine kinase activity that belongs to the family of epidermal growth factor receptor [36]. Overexpression of HER-2/neu which occurs in 20–30% of human breast cancers makes non-neoplastic mammary epithelial cells undergo malignant transformation [37]. Regardless of the animal species, promoter regions of the *cox-2* genes contain 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 [38]. Depending on the stimulus and the cell type, these transcription factors can modulate the expression of *cox-2*. Mouse *pea3* (the human gene is named *E1A-F* and *ETV4*) is the founding member of the *pea3* subfamily of *ets* genes, which also include *er81* (*ETV1*) and *erm* (*ETV5*) [39]. Ets proteins normally activate transcription of *cox-2*, but some members of the same family repress this process. In addition, Ets proteins play key roles in embryonic development, and their mutation and/or overexpression are associated with multiple malignancies [40]. PEA3 is up-regulated in Wnt1-expressing mouse mammary epithelial cells, and also in tumors from Wnt1 transgenic mice in which COX-2 is also up-regulated [41]. NFAT is a multi-component transcription factor regulating expression of several cytokine genes in antigen-activated T cells and functions as a major molecular target of the immunosuppressive drugs cyclosporin A. The NFAT family consists of at least five isoforms: NFAT1,

NFAT2 (NFATc), NFAT3, NFAT4, and NFAT5 [42]. In mesangial cells, activated NFAT2 by endothelin-1 upregulated *cox-2* gene expression [43]. In the Jurkat human leukemic T cell, cotransfection of NFAT wild-type protein transactivated the COX-2 promoter activity, and dominant negative mutants of NFAT2 protein abrogated COX-2 induction [44]. CRE was found to be involved in COX-2 induction by bradykinin in human pulmonary artery smooth muscle cells [45], and in TPA mediated differentiation of the human monocytoid U937 cells [46]. Mutation of the CRE site located in the COX-2 promoter significantly repressed COX-2 reporter induction by v-Src, platelet-derived growth factor, and serum in murine fibroblasts [47,48]. Recently, the histone acetyltransferase activity of CBP/p300 co-activator complex was found to be important for AP-1-mediated induction of *cox-2* [49] as schematically proposed in Fig. 2. In fibroblasts and macrophage, CBP/p300 was detectable in nucleus and its binding to a COX-2 promoter probe was enhanced by TPA, IL-1β or LPS. COX-2 promoter activities induced by these inducers were augmented by p300 overexpression [50].

Another plausible mechanism for modulating COX-2 expression involves PPAR activation. There are several different types of PPARs – α, β (or δ), and γ – and each acts as a transcription factor controlling gene expression as a complex with another nuclear protein, the retinoic acid receptor. Each PPAR affects expression of a different range of genes, most of which are generally involved in lipid metabolism [51]. Of the PPARs, PPARγ is of particular interest in mediating part of PG action because of the high potency of 15-deoxy-$\Delta^{12,14}$-PGJ_2 as an endogenous ligand for this nuclear receptor [52,53]. 15-Deoxy-$\Delta^{12,14}$-PGJ_2 significantly suppressed IL-1β-induced COX-2 expression and PGE_2 production in mesangial cells [54] and human

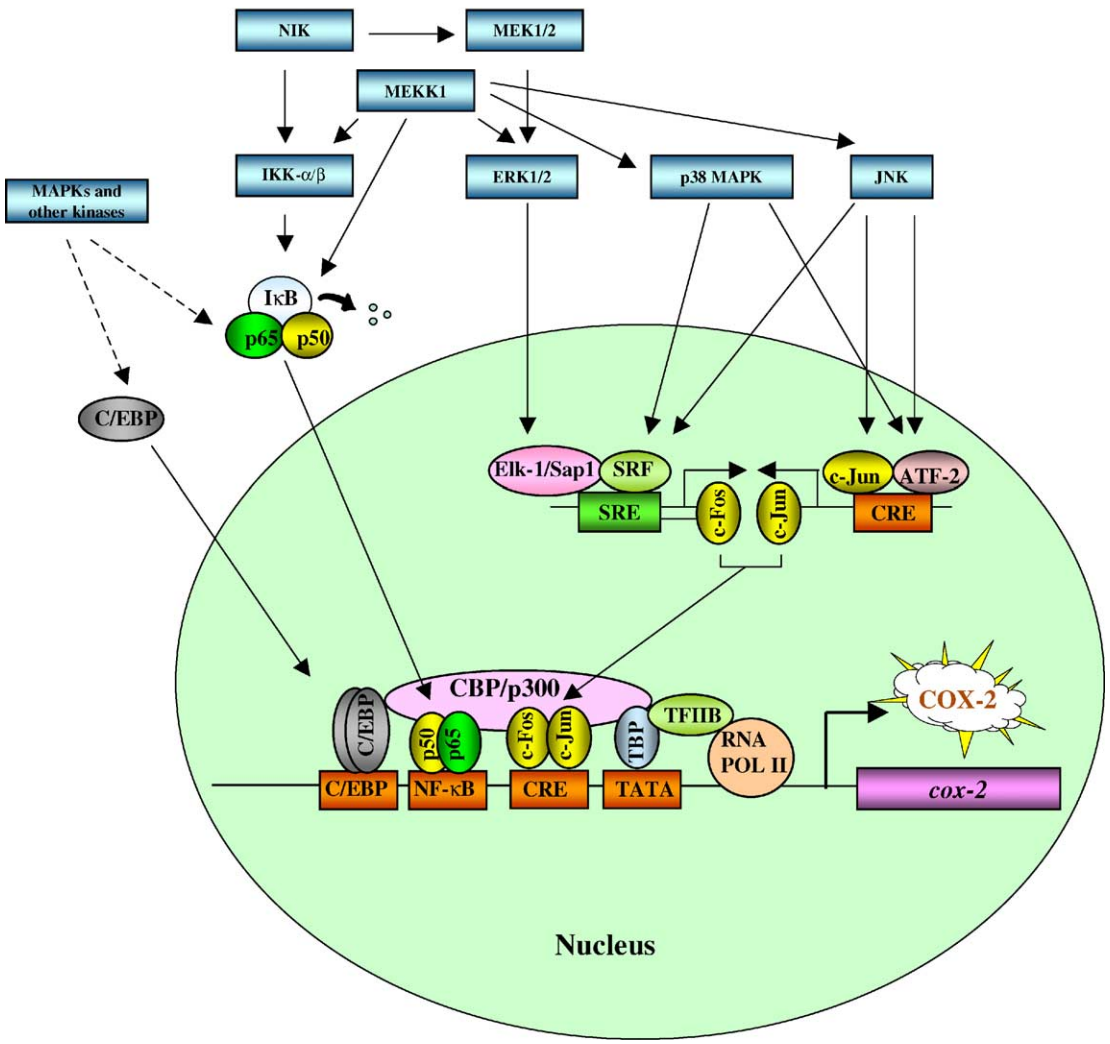

Fig. 2. Intracellular signaling pathways mediating COX-2 induction. A distinct set of upstream kinases including MAPKs can activate transcription factors (NF-κB, AP-1, C/EBP, etc.), which in turn leads to upregulation of COX-2.

astrocytes [55]. Activation of PPARγ by ciglitazone, a selective PPARγ ligand, resulted in apoptosis of HT-29 human colon cancer cells and suppressed expression of COX-2 [56]. In human vascular smooth muscle cells, activation of PPARα and PPARγ inhibited angiotensin II-stimulated COX-2 expression [57]. Recent studies also suggested that ligands of PPARγ stimulated the interaction between PPARγ and CBP, and overexpressing CBP partially reversed the inhibitory effects of PPARγ ligands on COX-2 expression [58]. On the other hand, PPARs have been found to increase COX-2 expression in corneal cells [59]. Likewise, activation of PPARδ resulted in increased *cox-2* mRNA and protein expression in human hepatocellular carcinoma [60], and PPARα activators also induced COX-2 expression in rat liver in vivo [61]. The regulation of COX-2 expression by PPARs is complex and appears to be cell-specific. The *p53*, a major tumor suppressor gene involved in the control of cell cycle progression, DNA integrity and cell survival, seems also to be involved in the regulation of COX-2 expression. A wild-type, but not a mutant form of p53 markedly suppressed transcription of *cox-2* [62]. Based on in vitro studies, p53 inhibited the binding of TATA-binding protein to the *cox-2* promoter region by competing with TATA-binding protein [62]. Leung et al. [63] reported that human gastric tumors with *p53* missense mutation exhibited a higher level of COX-2 expression when compared with tumors without *p53* mutation. These findings suggest that missense *p53* mutations may down-regulate the COX-2 expression.

3.1. NF-κB

Research over the last few years has revealed that NF-κB is an inducible and ubiquitously expressed transcription factor responsible for regulating the expression of genes involved in cell survival, cell adhesion, inflammation, differentiation, growth, etc. [64–67]. Active NF-κB complexes are dimers of various combinations of the Rel family of polypeptides consisting of p50 (NF-κB1), p52 (NF-κB2), c-Rel, v-Rel, Rel A (p65), and Rel B [68,69]. In most resting cells, NF-κB is retained in the cytoplasm by binding to one of the inhibitory IκB proteins (IκBα, IκBβ, IκBε, p105, and p100), which blocks the nuclear localization sequences of NF-κB [70]. NF-κB is activated in response to a wide variety of stimuli that promote the dissociation of IκBα through phosphorylation followed by ubiquitination, and degradation (Fig. 2). Thus unmasking of the nuclear localization sequence of NF-κB allows NF-κB to enter the nucleus and bind to κB-regulatory elements [71]. The phosphorylation of IκBα, as a critical event in NF-κB activation, is catalyzed by an IKK complex [72]. The core IKK complex consists of a heterodimer of IKK-α and IKK-β and two IKK-γ subunits. IKK-α and IKK-β mediate the phosphorylation of IκB, whereas IKK-γ links the core to the upstream signaling molecules [73]. In addition, another protein called IKK-complex-associated protein was found to interact with members of the IKK complex and is thought to act as a scaffold protein [74].

A growing body of evidence indicates that NF-κB plays a central role in general inflammatory as well as immune responses. The promoter region of COX-2 contains two putative NF-κB binding sites. Thus, NF-κB has been shown to be a positive regulator of COX-2 expression in murine macrophages [75] and human colon adenocarcinoma cell lines [76] exposed to LPS. In the former study, production of PGE_2 and 6-keto-$PGF_1\alpha$ in LPS-stimulated J774 macrophages was reduced by the antioxidant pyrrolidine dithiocarbamate and the serine protease inhibitor *N*-α-*p*-tosyl-L-lysine chloromethylketone which are inhibitors of NF-κB activation [75]. Liu et al. [77] have shown that pretreatment of rats with pyrrolidine dithiocarbamate suppressed LPS-induced NF-κB activation and also expression of COX-2. Expression of COX-2 in IL-1β-stimulated amnion mesenchymal cells was blocked by SN50, a cell permeable inhibitory peptide of NF-κB translocation, but not by SN50M, an inactive form of SN50 [78]. Many agents that activate MAPKs also activate NF-κB, suggesting that cross-talk occurs between these pathways (Fig. 2). MAPKs regulate NF-κB activation by multiple mechanisms. Accumulating evidence indicates that NF-κB activation is modulated by MAPK/ERK kinase kinase-1, a kinase upstream of JNKs [79] as well as p38 MAPK [80]. MAPK/ERK kinase kinase-1 induces site-specific phosphorylation of IκBα at Ser 32 and Ser 36 in HeLa cells and also directly activates the IKK complex [79]. Chen et al. [81] reported that vanadate treatment activated IKKβ and facilitated degradation of IκBα, which led to the activation of NF-κB through JNK activation in the murine macrophage cell line. Other studies showed that specific inhibitors of the ERK and p38 MAPK pathways blocked nuclear NF-κB activity and the transactivation activity of p65 [82–84]. Possible targets of these pathways include p65 itself, coactivators such as CBP/p300 and subunits of the RNA polymerase II complex (Fig. 2). Beyaert and colleagues [82] found that tumor necrosis factor α-induced phosphorylation of p65 was not modulated by the p38 inhibitor SB203580. In another study, inhibition of the p38 pathway significantly reduced the binding of TBP to the TATA box [85]. This may lead to reduced nuclear NF-κB activity by affecting the basal transcriptional complex rather than by directly affecting NF-κB itself. Therefore, one should be cautious in interpreting data obtained by using inhibitors of various signaling pathways that affect nuclear NF-κB activity unless the downstream targets are identified.

3.2. AP-1

Although AP-1 was identified as a transcription factor almost 15 years ago [86], and retroviral homologs of some of its components were identified even earlier [87], the biological relevance and physiological functions of AP-1

and its components still remain to be elucidated. The mammalian AP-1 proteins are homo- or heterodimers composed of basic region-leucine zipper proteins that belong to Jun (c-Jun, JunB and JunD), Fos (c-Fos, FosB, Fra-1 and Fra-2), Jun dimerization partners (JDP1 and JDP2) and the closely related activating transcription factors (ATF2, LRF1/ATF3 and B-ATF) subfamilies [88–90]. In addition, some of the Maf proteins (v-Maf, c-Maf and Nrl) can heterodimerize with c-Jun or c-Fos [91,92], whereas other Maf-related proteins, including MafB, MafF, MafG and MafK, heterodimerize with c-Fos but not with c-Jun [93,94]. Jun proteins can form stable dimers that bind AP-1 DNA recognition elements (5′-TGAG/CTCA-3′), also known as TREs based on their ability to mediate transcriptional induction in response to TPA [86]. Fos family proteins do not form stable dimers but can bind DNA by forming heterodimers with Jun proteins that are more stable than Jun–Jun homodimers [95,96]. ATF proteins, on the other hand, form homodimers as well as heterodimers with Jun proteins that preferentially bind to CRE (5′-TGACGTCA-3′).

Expression of the c-*fos* and c-*jun* is regulated by MAPKs (Fig. 2). The major MAPK-responsive element in the c-*fos* promoter is the SRE which is bound by a transcription factor complex including dimeric SRE and the ternary complex factors Elk-1, Sap1, and Sap2. The ERK, JNK, and p38 phosphorylate and activate Elk-1, resulting in enhanced SRE-dependent c-*fos* expression [97,98]. The predominant regulatory element in the promoter is TRE that preferentially binds to the heterodimer of c-Jun and ATF-2, both of which are activated by JNK. ATF-2 is also phosphorylated and activated by p38 [98]. c-Fos and c-Jun form heterodimer and bind to AP-1 response element in gene promoters. c-Fos is phosphorylated and activated by a novel protein kinase activity termed c-Fos-regulating kinase [99].

In support of the functional importance of AP-1 in COX-2 regulation, HER-2/neu-mediated activation of the *cox-2* promoter was suppressed by inactivating the CRE or overexpressing dominant negative c-Jun [49]. Xie and Herschman [100] showed that, in response to expression of v-Src or treatment with platelet-derived growth factor, c-Jun induced murine *cox-2* via the CRE site. Tumor-promoting phorbol ester also stimulated AP-1-mediated activation of COX-2 transcription via the CRE site [58]. Bombesin, a homolog of gastrin-releasing peptide, stimulated COX-2 expression through activation of ERK1/2 and p38 and increased activation and expression of the transcription factors Elk-1, ATF-2, c-Fos, and c-Jun [101]. These findings suggest that the upregulation of COX-2 by bombesin in intestinal epithelial cells can be stimulated via an AP-1-dependent pathway.

3.3. C/EBP

C/EBP transcription factors are also involved in regulating the activity of the COX-2 promoter. There is a C/EBP binding site on the human *cox-2* promoter at positions −132/−124, downstream of an adjacent AP-2 site, which plays a role in the induction of COX-2 by TPA and LPS [102]. The three main members of the C/EBP family include C/EBPα, C/EBPβ and C/EBPδ, which all recognize the same DNA sequence. They have a common structure, with an N-terminal domain bearing the transactivation sequence, a basic DNA-binding domain and a C-terminal domain containing a leucine zipper that allows the homo- or heterodimerization of these factors [103].

It is hard to generalize the functions of C/EBPs in the regulation of COX-2 transcription because their expression is quite cell type- and differentiation stage-specific. Even the same C/EBP isoform displays opposite effects depending on the cell type [104,105]. C/EBPα is known to be involved in the regulation of cell proliferation and differentiation. Many studies have shown that C/EBPα can induce growth arrest in various cell types. For example, C/EBPα expression is reduced in preneoplastic nodules compared with the surrounding normal liver tissues [106]. C/EBPα overexpression inhibits hepatocyte proliferation and also suppresses colony growth in mouse fibroblasts [107,108]. Furthermore, human hepatoma cell lines transfected with an inducible C/EBPα expression vector showed decreased tumorigenicity [109]. Unlike C/EBPα, C/EBPβ and C/EBPδ act primarily to regulate genes involved in mediating inflammation and cell proliferation and tend to be upregulated during the acute phase response [110]. Many studies showed either the transition of protein binding from C/EBPα to C/EBPβ and δ or a change in the relative expression level between C/EBPα mRNA and C/EBPβ and δ messages when genes are activated. For instance, in mammary epithelial cells and hepatocytes primed by the agents causing acute phase response, C/EBPβ and δ were predominantly expressed, whereas the level of C/EBPα was quite low [111,112]. In contrast, while the C/EBPα level increased, C/EBPβ and δ levels were down-regulated in terminally differentiated adipocytes and normal hepatocytes [113]. In mouse skin carcinogenesis, overexpression of COX-2 correlated with the increase in the C/EBPδ level and decreased C/EBPα expression [114]. Recently, Gorgoni et al. [115] demonstrated that *cox-2* mRNA induction and promoter activity were profoundly impaired in C/EBPβ (−/−) macrophages and could be rescued by expression of C/EBPβ. In contrast, COX-2 induction was completely normal in C/EBPβ-deficient fibroblasts, thus highlighting the diversity of cell-specific molecular mechanisms in determining inducible COX-2 expression.

Phosphorylation of C/EBPβ by MAPKs also increases the transactivating ability of this transcription factor. In NIH 3T3 cells [116] and 3T3-L1 preadipocytes [117], C/EBPβ was reported to be activated by phosphorylation at a threonine residue catalyzed by MAPK/ERK and SAPK2/p38. Cho et al. [118] found that JNK1(−) transfection abrogated both enhanced C/EBP DNA binding and C/EBPβ nuclear translocation by ceramide.

3.4. MAPKs

MAPKs phosphorylate specific serine and threonine residues of target protein substrates and regulate cellular activities including gene expression, mitosis, movement, metabolism, and programmed death [119]. Because of the many important cellular functions controlled by MAPKs, they have been subjected to exteneice investigation to define their roles in maintaining homeostasis or implications in human disease. MAPK-catalyzed phosphorylation functions as a switch to turn on or off the activity of their target proteins. Substrates of MAPKs include other protein kinases, phospholipases, transcription factors, and cytoskeletal proteins. Protein phosphatases remove the phosphates that were transferred to the protein substrate by the MAPK [120]. In this manner, the action of MAPKs and protein phosphatases reciprocally and rapidly alter the behavior of cells as they respond to changes in their environment. MAPKs serve as phosphorylation substrates for MAPK kinases. MAPK kinases-catalyzed phosphorylation activates the MAPK and increases its activity in catalyzing the phosphorylation of its own substrates. In multicellular organisms, there are three well-characterized subfamilies of MAPKs. These include ERKs (ERK1 and ERK2), JNKs (JNK 1, JNK 2, and JNK 3), and the four p38 enzymes (p38α, p38β, p38γ, and p38δ) [120].

It has been shown that MAPKs are important regulators in signaling pathways leading to proto-oncogene expression [121–123]. The activated MAPKs may translocate to the nucleus, where they phosphorylate the target proteins [124]. Recently, there is evidence indicating that MAPK family members play a role in *cox-2* gene expression induced by hypertonic saline solution and LPS [125]. Lo [126] reported that the MEK1/2 inhibitor PD98059 or the p38 inhibitor SB202190 attenuated LPS-induced *cox-2* mRNA expression as well as PGE_2 production in a concentration-dependent fashion in RAW 264.7 cells. In human keratinocytes, SB202190 attenuated UVB-induced *cox-2* mRNA expression, but PD98059 failed to significantly alter the COX-2 levels [127]. U0126, an ultrapotent initiator of the MEK1/2, blocked PGE_2 production and COX-2 mRNA expression induced by LPS in monocytes [128]. In a variety of cells treated with different inducing agents, specific inhibitors of p38 blocked the accumulation of *cox-2* mRNA [129,130]. In HeLa cells stimulated with IL-1 and in primary human monocytes stimulated with bacterial LPS, inhibition of p38 resulted in a rapid and specific destabilization of *cox-2* mRNA but had little effect on *cox-2* transcription [131]. Fig. 2 illustrates the MAPK-mediated signaling cascades leading to COX-2 induction.

4. Chemoprevention with selective COX-2 inhibitors: celecoxib as an example

Many of the side effects (e.g., gastrointestinal ulceration and bleeding, platelet dysfunctions, etc.) of NSAIDs have been ascribed to the suppression of COX-1-derived prostanoid production, whereas inhibition of COX-2-dependent PG synthesis accounts for the anti-inflammatory, analgesic, and antipyretic effects of these drugs. Consequently, the notion that specific inhibition of COX-2 provides therapeutic effects similar to those of NSAIDs without causing the unwanted side effects was the rationale for the development of selective COX-2 inhibitors as a new class of anti-inflammatory and analgesic agents with improved gastrointestinal tolerability.

Celecoxib (Celebrex™) is the first COX-2-specific drug approved for use to treat the signs and symptoms of adult rheumatoid arthritis and osteoarthritis. Celecoxib was recently 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 [132]. In this study, administration of 400 mg of celecoxib twice a day for 6 months caused a significant reduction in the burden of colorectal polyps on endoscopy. Celecoxib has also been shown to inhibit experimentally-induced tumorigenesis in several animal models (Table 1), including azoxymethane-induced colon tumorigenesis in F344 rats [133], DMBA

Table 1
The chemopreventive effects of celecoxib on experimentally-induced carcinogenesis

Animal	Inducer/treatment	Tissue
Rat	Azoxymethane	Colon [133,156]
	DMBA	Breast [134]
	DMBA	Mammary tumor [135,157,158]
	MNNG	Stomach [159]
Rat and mouse	*N*-Butyl-*N*-(4-hydroxybutyl)-nitrosamine	Urinary bladder [136]
Mouse	UV	Skin [137,138,160,161]
	Human lung adenocarcinoma cells implanted	Lung [162]
	Human osteosarcoma/rhabdomyosarcoma injected	Xenograft [163][a]
	Knock-out of trefoil factor 1 (TFF1)	Gastric tissue [164]
	Genetic mutation of *Apc* gene	Intestine [165]
Hamster	*N*-Nitroso-bis(2-oxopropyl)amine (BOP)	Pancreatic ductal cancer [166]

[a] Celecoxib administered in combination with doxycyclin in this study.

model of breast cancer in female rats [134], DMBA-induced rat mammary carcinogenesis [135], *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in male $B_6D_2F_1$ mice and female Fischer-344 rats [136]. Fischer et al. [137] reported that dietary celecoxib had a significant chemopreventive activity against UV-induced skin carcinogenesis in hairless SKH-1 mice. Pentland et al. [138] have also shown that orally administered celecoxib blocks the promotion stage of photocarcinogenesis in hairless mice. Recently, we have provided mechanistic basis for the anti-tumor promoting effect of celecoxib by addressing the role of AP-1 activation in TPA-induced COX-2 expression in mouse skin via the p38 MAPK pathway [139]. In this study, topical application of celecoxib suppressed expression of c-Jun and c-Fos protein induced by TPA. To determine which MAPKs play(s) a role in activation of AP-1 in mouse skin, we investigated the effects of the MEK1/2 inhibitor U0126 and the p38 inhibitor SB203580 on AP-1 activation. While SB203580 inhibited effectively the induction of AP-1 binding activity through inhibition of c-Jun expression, U0126 had no effect on AP-1 activation despite its ability to inhibit c-Fos expression. These results imply that c-fos may not be the key protein responsible for mediating the activation of AP-1 in TPA-treated mouse skin. In contrast, celecoxib failed to inhibit TPA-induced activation of NF-κB and nuclear translocation of its functionally active subunit p65 in our mouse skin model (data not shown). Niederberger et al. [140] recently showed that high concentrations of celecoxib, unlike other NSAIDs, did not inhibit but rather activated NF-κB and induced NF-κB-dependent gene transcription. Zweifel et al. [141] reported that inhibition of COX-2 by celecoxib resulted in loss of intratumor PGE_2 levels and reduced 1483 human head and neck xenograft tumors growth in a dose-dependent manner. Inhibition of COX-2 by celecoxib delayed tumor growth and metastasis in xenograft tumor models as well as suppressed basic fibroblast growth factor 2 induced neovascularization of the rodent cornea [142].

Celecoxib caused a dose- and time-dependent decrease in ODC activity in rat hepatoma HTC-IR cells [143]. ODC is the rate-limiting enzyme in the polyamine biosynthesis [144]. High levels of polyamines are usually correlated with rapid proliferation of cells, suggesting that ODC should be a critical target of chemopreventive agents. In the above study, celecoxib significantly decreased the expression of Egr-1, and dramatically reduced the levels of *c-fos* mRNA. Celecoxib and the ODC inhibitor difluoromethylornithine in combination elicited a strong therapeutic activity against UV-induced skin tumors in mice [145]. The mechanisms underlying difluoromethylornithine- and celecoxib-induced tumor regression remain to be clarified.

Celecoxib was shown to induce apoptosis in various cell lines via multiple mechanisms. Hsu et al. [146] showed that celecoxib-induced apoptosis in LNCaP and PC-3 cells independent of Bcl-2. In that study, celecoxib inhibited the phosphorylation of Akt/protein kinase B thereby blocking its anti-apoptotic activity. The effects of celecoxib on Akt were independent of phosphatidylinositol-3-kinase activity and were not reversed by okadaic acid, which is an inhibitor of protein phosphatases 1 and 2A, suggesting that this selective COX-2 inhibitor exerts a direct effect on Akt. Celecoxib inhibited the G_0/G_1 to S phase transition by decreasing the expression of cyclins and increasing the expression of the cell cycle inhibitory proteins $p21^{Waf1}$ and $p27^{Kip1}$ [147]. Celecoxib and *N*-(9-fluorenyl-methyloxycarbonyl)-L-leucine, a PPARγ agonist, significantly reduced the incidence and the multiplicity in rat mammary tumor to a greater extent than those achieved with individual treatments [148]. Celecoxib and *N*-(9-fluorenyl-methyloxycarbonyl)-L-leucine each down-regulated the cyclin-dependent kinase 1 and proliferating cell nuclear antigen expression in the tumor [148]. Celecoxib reduced the proliferation of COX-2-deficient HCT-15 colon cancer xenografts in nude mice, but had no significant anti-proliferative effect towards HT-29 tumors, which express COX-2 constitutively [147]. Maier and colleagues have provided additional evidence that apoptosis-inducing effects of celecoxib partly depend on COX-2 expression of Caco-2 cells transfected with the human COX-2 cDNA, whereas induction of a cell cycle block occurred COX-2 independently [149,150]. Several celecoxib derivatives, although lacking COX-2 inhibitory activity, were as potent in eliciting apoptosis in PC-3 cells as the parent compound [151]. Thus, the celecoxib may exert anticarcinogenic and chemopreventive activities by both COX-2-dependent and -independent mechanisms.

5. Conclusion and prospects

Since improper and abnormal overexpression of COX-2 is implicated in the pathogenesis of various types of human cancers, this inducible enzyme may be a useful surrogate biomarker for the evaluation of chemopreventives including NSAIDs. Pharmacologic inhibition of COX-2 reduces the formation of intestinal, esophageal, tongue, breast, skin, lung and bladder tumors in animals [29,136,137,152–156], which supports the notion that selective COX-2 inhibitors might be useful for preventing cancer.

Celecoxib, the selective COX-2 inhibitory drug, has been reported to reduce the formation of polyps in patients with familial adenomatous polyposis. This specific COX-2 inhibitor also protects against experimentally-induced carcinogenesis. However, the exact molecular mechanisms that account for the chemopreventive effects of celecoxib are not fully elucidated yet, and it is still controversial whether or not its anti-tumorigenic effects are mediated predominantly through suppression of COX-2 and subsequent PG synthesis. The precise mechanisms of action of celecoxib should be defined before wide application of this

drug for cancer intervention trials. With further progress in understanding the molecular biology of COX-2 and its clinical implications, the therapeutic as well as chemopreventive potential of celecoxib and other COX-2 specific NSAIDs is likely to expand.

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