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Review Article

The role of nonsteroidal antiinflammatory drugs in cancer chemoprevention

Charles Y.F. Young

Departments of Urology, and Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Mayo Clinic/ Foundation, Rochester, Minnesota, 55905, USA; e-mail: young.charles@mayo.edu

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Abstract

Epidemiological and laboratory studies, as well as human clinical intervention trials, strongly support a protective role for nonsteroidal antiinflammatory drugs (NSAIDs) in preventing the development of cancer. For example, NSAIDs are effective for reducing existing polyps in patients with familial adenomatous polyposis and reduce the tumor burden in several animal models of colorectal cancer. Many studies suggest that cyclooxygenase, especially COX-2, can be causally connected to carcinogenesis via modulation of the synthesis of prostaglandins and other eicosanoids, and subsequent effects on cell proliferation, angiogenesis, tumor growth, immune responsiveness and inflammatory reactions. It is therefore reasonable to assume that NSAIDs as inhibitors of COX-2 would be good candidates for cancer prevention, or even treatment. Animal studies and *in vitro* cell culture bioassays further demonstrated that cyclooxygenase-independent actions of NSAIDs could also contribute to their anticancer activities. These studies showed that NSAIDs can affect the expression and functions of many transcription factors related to oncogenesis, including hypoxia-inducible factor-1 (HIF-1), GATA-6, NF-κB, β-catenin/TCF-4, early growth response-1 (Egr-1) and the androgen receptor.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) have been widely used as antiinflammatory, antipyretic and pain-relieving (or analgesic) medicines, and some can even be purchased for regular use without a prescription. Evidence accumulating over the past three decades strongly suggests that NSAIDs may be useful for protecting from the development and/or progression of several types of cancer, including breast, lung, colorectal, esophageal and prostate cancer. Since cyclooxygenase type 2 (COX-2) has been causally connected to carcinogenesis, it is reasonable to propose that NSAIDs as inhibitors of COX-2 would be good candidates for use in cancer prevention, or even treatment (1-10).

Results from epidemiological studies, laboratory tests and clinical trials strongly support a protective role for NSAIDs against cancer development (11-18). Several epidemiological studies have shown a significant inverse association between the intake of aspirin and other NSAIDs and the risk of colorectal cancer in the general population (3, 11-15). Furthermore, NSAIDs are effective for reducing existing polyps in familial adenomatous polyposis and reduce the tumor burden in several animal models of colorectal cancer.

A study based on an analysis of 1,392 confirmed cases of breast cancer in a population of 80,741 postmenopausal women between the ages of 50 and 79 years, with no history of breast cancer or other cancers, was performed to evaluate breast cancer risk factors and NSAID use (10). The results showed that regular NSAID use for 5-9 years or for 10 years or longer was associated with a reduction of 21% and 28%, respectively, in the incidence of breast cancer. Interestingly, the study showed that the long-term use of ibuprofen provided a greater risk reduction than aspirin, whereas acetaminophen or low-dose aspirin (< 100 mg) was not effective in reducing the incidence of breast cancer.

Esophageal carcinomas are associated with a high mortality rate, making chemopreventive agents desirable. A meta-analysis of 9 studies (2 cohort, 7 case-control) including 1,813 cases assessed the relationship between aspirin/NSAID use and esophageal cancer (9). The

findings showed that both aspirin and nonaspirin NSAIDs had protective effects against esophageal adenocarcinoma and squamous cell carcinoma, more frequent use producing greater protection.

A similar inverse association has been described for other cancers, including ovarian and lung cancers. For prostate cancer, there are a few studies showing a weak, inverse association (not statistically significant) between NSAID use and cancer (19, 20). However, a recent report by Nelson and Harris (21) indicated a 70% reduction in the risk of prostate cancer among NSAID users. A more recent, community population-based study (18) at the Mayo Clinic showed a significant reduction in prostate cancer risk among men aged 60 or older who were daily users of NSAIDs. Interestingly, the study found the strongest inverse association among men aged 70 or older, with an 83% reduction in risk. The authors attributed this to the greater use of NSAIDs in this group of men.

Potential mechanisms for NSAID-mediated cancer chemoprevention

Cyclooxygenases and carcinogenesis

Although the mechanism(s) by which NSAIDs exert anticancer activity are far from completely understood, increasing evidence indicates that inhibition of arachidonic acid (AA) metabolic pathways via cyclooxygenase may contribute to the anticancer activity of NSAIDs (1-10). Cyclooxygenases can modulate the synthesis of prostaglandins and other eicosanoids that affect cell proliferation, angiogenesis, tumor growth, immune responsiveness and inflammatory reactions (11-12, 14, 15). Cyclooxygenase enzymes may be involved in activation of carcinogens and the formation of reactive oxygen species (11). Thus, NSAIDs that inhibit cyclooxygenase enzyme activities may be potential chemopreventive agents.

There are two cyclooxygenase genes, *COX-1* and *COX-2,* located on chromosomes 9 and 1, respectively; COX-1 is constitutively expressed in many tissues for normal physiological functions and COX-2 is inducible by cytokines in mediating inflammation (3, 11). Both COX-1 and COX-2 share about 60% homology in amino acid sequence. The active site of these isozymes only differs at two positions. In fact, both isozymes have almost identical catalytic kinetics, causing oxygenation of arachidonic acid to produce PGG_2 and PGH_2, and subsequent prostaglandins and thromboxane by separate enzymes depending on cell or tissue type. However, COX-2 may be able to use more alternative substrates than COX-1, *e.g.*, COX-2 can use docosahexaenoic acid and endocannabinoids as substrates. As mentioned above, both COX-1 and COX-2 have the same substrate specificity, and specific inhibitors with clinically demonstrable selectivity for the isozymes have been developed and marketed.

Interestingly, the finding that the potent antipyretic and analgesic effects of acetaminophen could not be explained by either COX-1 or COX-2 blockade eventually led to the discovery of COX-3, a splice variant of COX-1, in canine brain (22-24). The COX-3 variant is sensitive to acetaminophen and considered to play a critical role in the biosynthesis of prostanoids known to be important mediators in pain and fever. Drugs that preferentially block COX-1 also appear to act at COX-3. In addition, other splice variants of COX-1 have been described and may be detectable in human tissues, although their significance requires further study. Moreover, the existence of COX-3 at the nucleotide sequence level in humans has not been firmly proven.

Several lines of evidence have been presented that link cyclooxygenase and its prostaglandin products to cancer development, and perhaps progression. The first clue was that cancer tissue prostaglandin levels are often correlated with poor postoperative survival or the development of metastasis (25, 26). Increased levels of prostaglandins, especially PGE_2, were found in many cancer tissues compared to their normal counterparts (27-31). Similar results were also found in cancer tissues from experimental animal models of carcinogenesis. It has been shown that PGE_2 can be synthesized in cancer cell lines. In cell culture systems, PGE_2 may enhance tumor cell proliferation.

In the early 1990s, COX-2 was shown to be one of the proteins induced by the viral oncogene *c-src* in cell transformation or by growth factors/receptors, including the EGF (epidermal growth factor) receptor, the IGF-1 (insulin-like growth factor) receptor, the heregulin/HER-2 receptor or mutations in APC and *ras* (6, 8, 32, 33). The COX-2 isozyme has been shown to be overexpressed in many tumor tissues and cancer cell lines (27-31). Overexpression of COX-2 via transfection was associated with increases in PGE_2 and Bcl-2, and a subsequent decrease in apoptosis (8). Recently, a transgenic model demonstrated that overexpression of COX-2 alone is enough to cause mammary tumorigenesis. Furthermore, chemically induced mammary tumorigenesis in rats can be prevented by the selective COX-2 inhibitor celecoxib. It thus appears that COX-2 may be more relevant than COX-1 to tumor development.

However, some studies indicated that COX-1 could also have a role in carcinogenesis. COX-1 knockout Min mice showed an 80% reduction in the development of polyposis (34). A more recent study (35) seemed to suggest that COX-1 constitutively expressed in stromal fibroblasts and providing basal levels of PGE_2 could be critical for the initial establishment of polyps. In both animal and human tissues, COX-1 and COX-2 were found to be co-expressed in fibroblast cells in polyps, although the expression of COX-2 is induced when polyps grow to over 1 mm in size. COX-2 may produce more PGE_2 for boosting tumor growth. Furthermore, as suggested in some studies, one intriguing aspect is that increasing COX-2 activities with increased PGE_2 may be related to immunosuppressed states of cancer, resulting from

increasing IL-10 and decreasing IL-1, IL-12 and tumor necrosis factor (TNF) levels (8). Of course, the inhibition of cyclooxygenase by NSAIDs resulting in reduction in cancer risk would prove the action of cyclooxygenase in carcinogenesis.

Furthermore, COX-2 can play an important role in tumor angiogenesis and invasion by modulating the expression and function of vascular endothelial growth factor (VEGF) and integrin-mediated signaling via COX-2 enzymatic products (1, 6, 8). Angiogenesis is a hallmark of tumor expansion and metastasis. In the early 1970s, it was suggested that the new capillary blood vessels (or microvessels) are necessary for cancer cells to grow if tumor mass reaches 1-2 mm in diameter, because at that point the supply of the nutrients, including oxygen, can not reach those tumor cells distant from the nearest vessels. Only those tumors capable of stimulating and developing new microvessels may have the potential to become metastatic.

There are two types of cells, *i.e.*, endothelial cells and pericytes, in vessels that can respond to proper stimuli to form new vessels. Normally these cells are quiescent, with a life span of several years. However, many angiogenic or proangiogenic agents can stimulate these cells, including those from tumor cells. Once the proangiogenic growth factors diffuse from tumor cells to preexisting blood vessels, quiescent enthothelial cells will be activated and induce a cascade of events, including proteolytic breakdown of the basement membrane, migration of the endothelial cells towards the angiogenic sources and proliferation of the cells, lumen formation, pericyte capping, and finally, formation of a new basement membrane for the new microvessels.

Cyclooxygenases, especially COX-2, have been suggested to be directly related to the angiogenesis of tumors. As mentioned above, catalytic activities of increased COX-2 will enhance the production of eicosanoids, including thromboxane A_2 (TxA_2), PGE_2 and PGI_2, which are essential for neovascular formation, providing sufficient nutrients for subsequent tumor growth. Thromboxane A_2 also plays a critical role in COX-2-dependent endothelial migration and neovascularization of tumors (1, 6, 8). It has been shown that migration of the endothelial cells can be inhibited by a COX-2-selective inhibitor via a reduction in TxA_2 levels. Adding exogenous TxA_2 to the cells can reverse the effects of the COX-2-selective inhibitor. Overexpression of COX-2 appears to be responsible for the production of many proangiogenic molecules, such as VEGF, basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), as well as integrin-mediated signaling (1, 6, 8). Metalloproteinases may be upregulated by COX-2 via PGE_2 in prostate and colon cancer cell lines.

Prostaglandins as cyclooxygenase metabolic products exert their biological actions through binding to their plasma membrane receptors (prostanoid receptors) with seven transmembrane domains (36). Prostaglandin-activated receptors appear to act via G-protein to cAMP and protein kinase A to increase VEGF. Studies using prostanoid receptor knockout mice strongly suggested that the prostanoid receptor signal is important for tumor-associated angiogenesis and subsequent tumor growth. Experiments in prostanoid receptor knockout mice bearing tumor xenografts showed that receptors in the host stroma could play a critical role in tumor-associated angiogenesis. The studies indicated that antagonizing prostanoid receptors on host stromal cells is more effective than inhibiting the receptors in tumors for preventing tumor growth. Developing antagonists for prostanoid receptors may therefore represent a new approach to cancer prevention.

Specific COX-2 inhibitors vs. nonselective cyclooxygenase inhibitors

Chronic use of traditional NSAIDs which block both cyclooxygenase enzymes may cause serious side effects such as gastrointestinal bleeding, ulceration and renal toxicity (3, 11, 14, 15). The side effects of traditional NSAIDs are due mainly to inhibition of COX-1. As mentioned above, COX-1 is generally constitutively expressed in many tissues, whereas COX-2 is inducible by proinflammatory cytokines (3, 11). Importantly, COX-2 has been found to be overexpressed in many tumor tissues (3, 15). Therefore, improved, new-generation NSAIDs are designed to target COX-2 and thereby reduce the above side effects (3, 14, 15).

Large randomized trials have recently been performed evaluating long-term use of NSAIDs, including the Vioxx Gastrointestinal Outcome Research and the Celecoxib Long-Term Arthritis Safety Study (CLASS) (37, 38). These studies appeared to show that the COX-2-selective NSAIDs are associated with an improved gastrointestinal safety profile compared to the classical NSAIDs. However, a cautionary note has been raised in a study regarding the potential cardiovascular risk associated with the use of the NSAIDs (39). Another unexpected result from more recent studies suggested that aspirin might increase the risk of human pancreatic cancer (40, 41), whereas previous studies demonstrated that NSAIDs can decrease experimental pancreatic carcinogenesis in hamsters (42). A previous human survey also showed that frequent use of aspirin may reduce the risk of pancreatic cancer (43). *In vitro* data indicated antineoplastic effects for NSAIDs on pancreatic cells (44). However, the latest study (40) suggested that animal models and *in vitro* studies may not be very relevant to humans. Clearly, more studies should be performed to clarify the issue.

NSAIDs and cyclooxygenase in cancer prevention

Cyclooxygenase dependence

Recently, it has been proposed that activation of inducible nitric oxide synthase (iNOS) and consequent

generation of NO may be associated with the development of cancer in a variety of gastrointestinal diseases. Furthermore, both COX-2 and iNOS have been reported to be implicated in breast and colon cancer progression (45, 46). Interestingly, another study showed that PGE_2 generated from COX-2 expression in the highly metastatic murine breast cancer cell line C3L5 can upregulate interferon gamma + lipopolysaccharide (LPS)-induced iNOS expression and NO production (47). It was further demonstrated that upregulation of iNOS by PGE_2 is mediated by the PGE_2 EP_4 receptor in a cAMP-dependent manner. The increasing levels of NO can be suppressed by NSAIDs, which is reversible by exogenous PGE_2 or the EP_1 receptor agonist PGE_1 alcohol.

As discussed above, it is generally agreed that the chemopreventive function of NSAIDs may be due to their cyclooxygenase-inhibitory effect on prostaglandin production. Furthermore, it was reported that NSAIDs such as sulindac and indomethacin could induce apoptosis in colon cancer cells by accumulating a relative high concentration of arachidonic acid, which in turn stimulated the conversion of sphingomyelin to ceramide, a known mediator of apoptosis (48). Accumulation of arachidonic acid by inhibiting its conversion to PGE_2 can therefore activate sphingomyelinase, leading to an increase in ceramide. This effect may not be restricted to colon cancer cells. It could also be observed in primary fibroblasts and immortalized keratinocytes. Thus, this effect might have general application to NSAID-mediated cancer prevention.

It has been known for many years that high serum estrogen levels may increase the risk of breast cancer (2, 49-52). Recently it was shown that aromatase (CYP19) can be detected in breast tumor tissues and may catalyze the production of estrogens in the tumor tissues (53-55). Intratumor aromatase and estrogen production may have a role in breast cancer development and progression. The expression of aromatase in normal and cancerous breast tissues may be differentially regulated. A so-called promoter switch theory has been proposed in which cAMP may be a critical factor causing alternative promoter activation in the aromatase gene. PGE_2 produced by COX-2 and certain cytokines can upregulate the expression of aromatase (56, 57). Interestingly, it was demonstrated using a semiquantitative RT-PCR assay (58) that COX-2 and aromatase were both overexpressed in 23 human breast cancer tissues. This finding seems to indicate that selective NSAIDs targeting COX-2 to reduce PGE_2 and, subsequently, aromatase activity would be an appealing strategy for reducing breast cancer risk, and potentially other hormone-related cancers.

Several transcription factors can be regulated by NSAIDs. These factors may be related to cell proliferation and angiogenesis. In ischemic conditions, the HIF complex is activated and binds a hypoxia response element in the promoters of the *VEGF* gene and the VEGF receptor Flt-1 (59-66). The HIF complex consists of a heterodimer of HIF-1α and an aryl hydrocarbon receptor nuclear translocator (HIF-1β). Under normoxic conditions, HIF-1β is relatively stable, but HIF-1α binds to the von Hippel-Lindau tumor suppressor protein (VHL) and becomes susceptible to rapid degradation via ubiquitination. On the other hand, under hypoxia, HIF-1α and HIF-1β form a stable complex and bind a hypoxia response element in the promoters of the *VEGF* and *Flt-1* genes onto which AP-1 proteins are recruited, followed by the initiation of transcription. It is known that hypoxia by over- and outgrown tumor tissue mass may trigger neovascularization in the tumor mass and help tumor cells grow and spread. NSAIDs can inhibit hypoxia-induced angiogenesis. Although it has been shown that NSAIDs decrease VEGF and Flt-1 by reducing HIF-1 expression via an increase in VHL levels and an increase in ubiquitination of HIF-1α, exactly how NSAIDs induce downregulation of HIF-1 is not clear. Since PGE_2 was shown to induce HIF-1α stability and nuclear localization, it is reasonable to assume that inhibition of PGE_2 production by NSAIDs may contribute to the downregulation of HIF-1α and its downstream products VEGF and Flt-1 (59-66).

Cyclooxygenase independence

The early growth response factor Egr-1 is another transcription factor that is important in angiogenesis induced by hypoxia (67-69). Egr-1 can regulate a number of genes, including *Flt-1* and epidermal growth factor (EGF) receptor. Vascular endothelial growth factor can induce Egr-1 expression, which in turn activates the expression of Flt-1 (68-72). Therefore, as mentioned above, VEGF expression could be affected by NSAIDs via inhibition of cyclooxygenase activity. In one study (73), NSAIDs such as indomethacin and the COX-2 inhibitor NS-398 were shown to be able to inhibit Egr-1 expression stimulated by exogenous VEGF in cultured human microvascular endothelial cells (HMVEC). Since the effective concentrations of the NSAIDs used to inhibit the expression of Egr-1 were much higher than those for repressing cyclooxygenase activity, the effect of NSAIDs in this case might be viewed as a cyclooxygenase-independent mechanism.

The APC/β-catenin/TCF pathway (Wnt signaling pathway) has been suggested to be involved in the initial stages of the adenoma-carcinoma sequence. Treatment with sulindac of 5 patients with familial adenomatous polyposis (FAP) for up to 6 months was found to reduce adenoma nuclear β-catenin expression when compared to pretreatment adenomas from the same patients (74). *In vitro* studies (75, 76) with sulindac sulfide, a sulindac metabolite lacking cyclooxygenase-inhibitory activity, in the colorectal cancer cell lines DLD-1 and SW480 showed decreased levels of nonphosphorylated β-catenin and a subsequent reduction in β-catenin-mediated transcription of the target genes *Met* and *cyclin D1*. This action of sulindac sulfide suggests a COX-2-independent mechanism in the chemopreventive effect of NSAIDs.

Moreover, several mechanisms have been proposed regarding how NSAIDs affect the transcriptional

function of β-catenin/TCF (76). For example, the downregulation of β-catenin may be caused by caspase- and proteasome-dependent degradation pathways, as a result of its enhanced phosphorylation (77) by glycogen synthase kinase-3β (GSK-3β) in SW948 and SW480 colorectal cancer cells (78, 79). On the other hand, it was shown that both aspirin and indomethacin can inhibit β-catenin/TCF-mediated transcription without affecting its levels (78, 79). In an animal study, sulindac, indomethacin, meloxicam and sulindac sulfone all effectively suppressed chemically induced rodent colorectal tumor formation and inhibited β-catenin nuclear translocation as assayed by immunohistochemical staining (75).

Interestingly, a new class of NSAID, *i.e.,* NO-donating aspirin, has been developed recently with much higher potency than the parent aspirin (80-82). This new chemical can inhibit NOS2 expression/NO formation, β-catenin/TCF-mediated transcriptional activity and NF-κB DNA binding ability at concentrations lower than or near those required for inhibiting cell growth or killing cells. Thus, NO-aspirin may act at critical early events in tumor formation as a chemopreventive agent in colon cancer.

It has been shown that NSAIDs can suppress cancer cell proliferation, soft agar colony formation and the induction of apoptosis regardless of the cyclooxygenase status of the cells (3, 83-85). Sulindac sulfone, a metabolite of the NSAID sulindac that lacks cyclooxygenase-inhibitory activity, blocked tumor growth in mice (16, 86). Moreover, sulindac, an inhibitor of COX-1 and COX-2, induced apoptosis by blocking the antiapoptotic protein Bcl-X and inactivating peroxisome proliferator-activated receptor PPARδ. Also, celecoxib was reported to downregulate the antiapoptotic protein Bcl-2 (85). However, another study (87) reported that the apoptosis induced was due to blockade of Akt activation independent of Bcl-2. Furthermore, aspirin, sulindac and sulindac sulfone all inhibited tumor cell proliferation by blocking activation of I-κB kinase- (IKK-β), which is required for activation of NF-κB (88). Active NF-κB enhances cell survival.

Moreover, NSAIDs can inhibit cyclooxygenase-independent angiogenesis (92). Recently, it has been shown (90) that sulindac downregulates the transcription repressor GATA-6 for the 15-lipoxygenase-1 (15-LOX-1) promoter and enhances the expression of 15-LOX-1, as well as the product of 15-LOX-1, 13-(*S*)-hydroxyoctadecadienoic acid (13-[*S*]-HODE), which in turn enhances the apoptosis of colorectal cancer cells. It has been shown that linoleic and arachidonic acid metabolites can be peroxisome proliferator-activated receptor (PPAR) ligands and the PPARδ can enhance cell survival and proliferation, and promote colonic tumorigenesis (91-96). Moreover, activated PPARδ can upregulate the expression of COX-2 (97-99). It was shown that 13-(*S)*-HODE can act as a PPARδ antagonist and decrease PPARδ activation, subsequently downregulating PPARδ expression and resulting in apoptosis in colorectal cancer cells. These studies seem to provide a mechanistic link through NSAID-induced 15-LOX-1 and its product 13-(*S)*-HODE to induction of apoptosis by downregulation of PPARδ. It is clear that NSAIDs possess cyclooxygenase-independent anticancer effects. However, *in vitro* experimental results should be interpreted cautiously, because many studies did not clearly define or can not judge whether the actions of the NSAIDs used are strictly cyclooxygenase-independent.

Recently, we found that NSAIDs may exhibit a new biological activity: suppressing the androgen receptor in prostate cancer cells (100, 101). Prostate cancer exceeds lung cancer as the most commonly diagnosed cancer in males and is the second leading cause of male cancer death in many western countries. The androgen receptor, a ligand-dependent transcription factor, plays a central role in androgen actions in the prostate, as well as being a risk factor for the development and progression of prostate cancer (102-109). A prospective cohort study demonstrated a strong positive association between plasma testosterone levels and prostate cancer risk (107). Removal or blockade of the synthesis of androgens, thereby inactivating androgen receptor activity, can result in the induction of apoptosis in both normal and cancerous prostatic epithelia *in vivo* (102, 110, 111). Males with 5α-reductase deficiency or castration at an early age do not develop benign or malignant prostate tumors (102, 103, 111). The above evidence provides strong support for androgen deprivation-based chemoprevention and chemotherapy for prostate neoplasia.

The androgen receptor is considered to be the central factor in the development and progression of prostate cancer. However, it has not clearly been explained how prostate cells are transformed and become malignant, although it is thought that growth stimulation (proliferation) can increase the number of mutated cells, which are further stabilized (survival) by androgens and their receptor. The transformation to malignancy is mainly induced by genetic changes in normal cells via environmental factors (mutagenic factors: chemical and physical) and endogenous factors (hormones, growth factors, etc.) (112-114). Recently, it was postulated that androgens and the androgen receptor could be potentially promutagenic. Overactivation of the androgen receptor due to high levels of androgens might produce excessive reactive oxygen species (ROS), which can be mutagenic and harmful (115, 116). The interaction with other environmental factors (dietary factors such as animal fat) can further enhance the transformation rates of prostate cells *in vivo*. Recently, overexpression of many cofactors (*e.g.,* caveolin-1, STAT3, β-catenin) or coactivators (*e.g.*, TIF2 and SRC-1) for the androgen receptor was suggested to enhance the function of the receptor at low levels of androgens or in the presence of other nonandrogen ligands (117-129). Of course, mutation and amplification of the androgen receptor can also enhance the function of the receptor in a similar way. Additionally, polymorphism in a trinucleotide repeat (CAG) encoding a polyglutamine stretch in the *N*-terminal domain of the androgen receptor has been associated with an increasing risk of prostate cancer (if the CAG tract is shortened) (130-132).

Although epidemiological studies suggest a negative association between consumption of NSAIDs and the risk

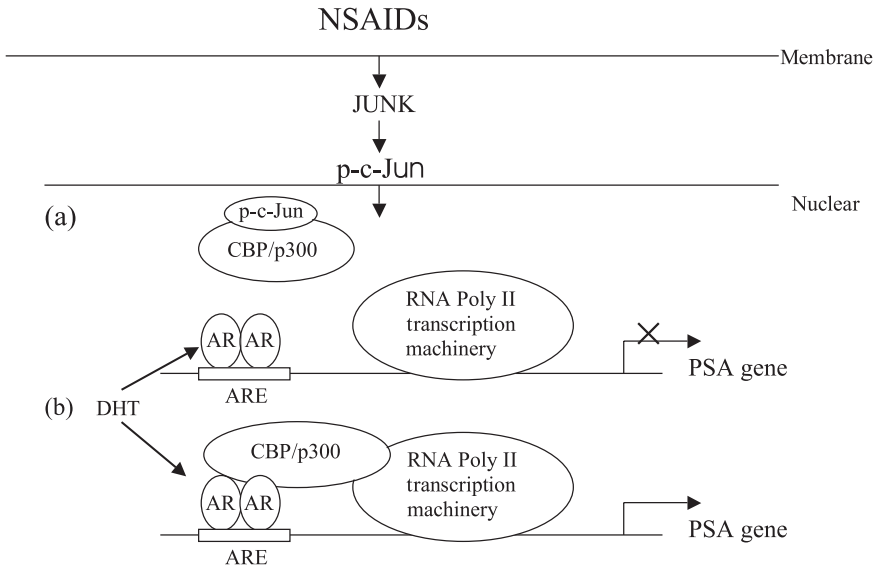

Fig. 1. Proposed mechanisms by which celecoxib and nimesulide may inhibit the function of the androgen receptor (AR) in prostate cells. (a) After treatment with the NSAIDs, JUNK is activated, which may cause phosphorylation of c-Jun (p-c-Jun) and consequently prolong overexpression of c-Jun protein. Prolonged overexpression of c-Jun could in turn sequester the coactivator CBP/p300 from the AR, thereby inhibiting its function even in the presence of androgens such as dihydrotestosterone (DHT). (b) Upon binding and activation by androgens such as DHT, the AR in the nucleus recruits CBP/p300 while binding to the androgen response element (ARE) of the promoter of androgen-regulated genes, including the prostate-specific antigen (PSA) gene. CBP/p300 can acetylate the AR and enhance its activity, and also acts as an integrator between the AR and basic transcription machinery for activation of the expression of androgen-regulated genes.

of prostate cancer, the COX-2 protein or its enzymatic activity is not frequently overexpressed in prostate cancer tissues (91, 133). This suggests that cyclooxygenase inhibition by NSAIDs may not be the sole mechanism for prostate cancer prevention. According to our recent studies, the COX-2 inhibitors celecoxib and nimesulide appear to have much higher potency than several other NSAIDs tested in inhibiting the expression and/or function of the androgen receptor. Thus, their *in vivo* effective concentrations may be achievable, in contrast to other NSAIDs. Modulating the androgen receptor may be one of the mechanisms by which these NSAIDs can inhibit the development of prostate cancer. This indicates that these two NSAIDs, and perhaps also their analogues, may be potential agents for prostate cancer prevention.

Our studies indicated that the NSAIDs could stimulate a prolonged overexpression of c-Jun (101). It was suspected that, in part, phosphorylation of c-Jun could stabilize and therefore prolong the expression of c-Jun. c-Jun is one of the components of activating protein-1 (AP-1) and a member of the basic leucine zipper (bZIP) family of sequence-specific dimeric DNA-binding proteins. It has been shown that Jun can control a highly diverse set of genes, many of which may be related to cellular proliferation processes (134-136). Recently, it was also demonstrated that Jun proteins may be related to cellular apoptosis and growth inhibition. Interestingly, c-Jun can act as a positive transcription factor or a repressor. It can bind CBP/p300, SRC-1 or TIF2/GRIP1 and other coactivators as a positive transcription factor (137-141). On the other hand, it can bind to steroid nuclear receptors and act as a repressor (137, 138). In different cases, c-Jun can interact with corepressors such as N-CoR, SMRT or TG-interacting factor to repress gene expression (137-141).

It has been shown that members of the AP-1 protein family are upregulated in the castrated prostate (142, 143). Previous results have shown that stimulated overexpression of c-Jun protein can inhibit the function of the androgen receptor (144-149). Several studies have shown that the transactivation functions of the androgen receptor, as well as other steroid receptors, can be affected by c-Jun (150-154). It was further demonstrated that there is a direct protein-protein interaction between the DNA- and ligand-binding domains of the androgen receptor and the leucine zipper region of c-Jun that might affect the function of the DNA-binding domain of the androgen receptor (153). Additional studies indicated that the transcriptional interference between the androgen receptor and AP-1 is partly mediated through competition for intracellular CBP/p300 (152, 154); CBP serves as an integrator/coactivator for androgen receptor transactivation activities. Further studies are required to ascertain whether c-Jun is indeed able to interfere with the interaction of CBP and the androgen receptor by NSAIDs (Fig. 1).

Bioactivity and bioavailability of NSAIDs

There are sufficient data demonstrating that the potential *in vivo* antitumor activities of NSAIDs are

separable from cyclooxygenase-inhibitory activity (155-158). However, many *in vitro* cell culture systems used to show antiproliferative or apoptosis-inducing activity of NSAIDs require higher concentrations than those inhibiting cyclooxygenase activity (158). The differences in the concentrations for these two different activities of the same NSAIDs may reach 100- to 1,000-fold. Usually, low micromolar concentrations of many NSAIDs are associated with inhibition of cyclooxygenase activity, whereas much higher concentrations of the same NSAIDs are required for *in vitro* cell growth inhibition or the induction of apoptosis. The high concentrations of NSAIDs could be toxic *in vivo* and may not be reached in the bloodstream, which could be a necessary safeguard. Few NSAIDs may be able to reach their antiproliferative dose in the circulation.

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