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Minireview paper

COX-2 inhibitors in cancer treatment and prevention, a recent development

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Epidemiological and experimental studies have demonstrated the effect of non-steroidal anti-inflammatory drugs (NSAIDs) in the prevention of human cancers. NSAIDs block endogenous prostaglandin synthesis through inhibition of cyclooxygenase (COX) enzymatic activity. COX-2, a key isoenzyme in conversion of arachidonic acid to prostaglandins, is inducible by various agents such as growth factors and tumor promoters, and is frequently overexpressed in various tumors. The contribution of COX-2 to carcinogenesis and the malignant phenotype of tumor cells has been thought to be related to its abilities to (i) increase production of prostaglandins, (ii) convert procarcinogens to carcinogens, (iii) inhibit apoptosis, (iv) promote angiogenesis, (v) modulate inflammation and immune function, and (vi) increase tumor cell invasiveness, although some studies indicated that NSAIDs have COX-2-independent effects. A number of clinical trials using COX-2 inhibitors are in progress, and the results from these studies will increase our understanding of COX-2 inhibition in both cancer treatment and prevention. The combination of COX-2 inhibitors with radiation or other anti-cancer or cancer prevention drugs may reduce their side effects in future cancer prevention and treatment. Recent progress in the treatment and prevention of cancers of the colon, esophagus, lung, bladder, breast and prostate with NSAIDs, especially COX-2 inhibitors, is also discussed. [© 2002 Lippincott Williams & Wilkins.]

Key words: Cancer prevention, cyclooxygenase, non-steroidal anti-inflammatory drugs.

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Introduction

Aspirin and its related drugs, also called non-steroidal anti-inflammatory drugs (NSAIDs), are the most widely used medicines around the world. Thousands of years ago, the ancient Egyptians, Greeks and Romans started to use salicylate-containing plants, such as the white willow, to relieve pain and reduce fever. Aspirin was first synthesized and introduced into clinical practice in the 1890s by Bayer, which soon became a household name around the world for the treatment of rheumatic fever and pain relief.[1] In the 1940s, aspirin was found to have anti-thrombotic effects and to prevent vascular events, which promoted its use in protection from myocardial infarction and blood clots.[1] However, the mechanisms of aspirin's actions on fever and pain and on blood coagulation were not discovered until 1971. Since publication of a series of papers by Vane *et al*.[2] and Smith and Willis,[3] we have understood that the analgesic, anti-inflammatory, anti-pyretic and anti-coagulation effects of aspirin arise from its inhibition of prostaglandin synthesis from arachidonic acid through selective inhibition of prostaglandin synthetase. Dr Vane received a Nobel Prize in 1982 for his achievement in elucidating the actions of this important drug.

More recently, epidemiological, experimental and clinical studies have demonstrated that aspirin and some other NSAIDs can decrease the incidence of colorectal,[4–8] esophageal,[9,10] lung[11–13] and bladder[14,15] cancers among regular users or in experimental animals. A new NSAID, celecoxib, was approved by the US Food and Drug Administration for the prevention of colorectal cancer in patients with familial adenomatous polyposis (FAP). Moreover, NSAIDs have been reported to help prevent or

delay the onset of Alzheimer's disease.[16,17] Taken altogether, this evidence supports the belief that the effects of NSAIDs are mediated mainly by their inhibition of prostaglandin synthesis.[18,19]

In mammals, two key enzymes that respond to prostaglandin synthesis have been characterized as cyclooxygenase (COX)-1 and -2; they are also called prostaglandin H synthetase (PGHS)-1 and -2, and are encoded by two different genes located on chromosome 9 and 1, respectively.[18,19] COX-1 is constitutively expressed in many tissues and is thought to be involved in the homeostasis of various physiologic functions; COX-2, its isoenzyme, is overexpressed in many different cancers,[18,19] and can be induced by various agents such as growth factors and tumor promoters.[20–25]

Because intestinal epithelial cells that overexpress the COX-2 gene have altered adhesion properties and resist apoptosis, it has been suggested that overexpression of COX-2 may be involved in colorectal carcinogenesis.[26] Further, these changes are reversed by treatment with NSAIDs.[26] COX-1, on other hand, has the important housekeeping property of maintaining a number of physiologic functions of the cell. Thus, selective inhibition of COX-2 enzymatic activity and/or its expression has become more and more important in clinical treatment and prevention of human cancer. The current approach to designing and evaluating selective COX-2 inhibitors has been to maximize their efficacy while largely eliminating their adverse side effects, such as gastrointestinal bleeding and peptic ulceration. A number of selective COX-2 inhibitors have been synthesized and evaluated both *in vitro* and *in vivo* for many indications.[27] This review, however, focuses only on the anti-tumor activities of certain NSAIDs.

COX-2 and carcinogenesis

Evidence for the involvement of COX in cancer development first came from pharmacological analyses of prostaglandins in different human cancers[28,29] because of their key roles in metabolism of arachidonate, which catalyzes the biosynthesis of prostaglandin H_2, the precursor for prostanoids.[18,19,26] Prostaglandin E_2 (PGE_2) exerts especially carcinogenic effects in the human body. Several studies have indicated that premalignant lesions and established cancers produce excessive quantities of PGE_2, and that administration of PGE_2 enhances tumor cell growth and increases tumor invasiveness.[28–31] Furthermore, a recent study by Williams *et al*. found that tumor growth was markedly attenuated in COX-$2^{-/-}$ mice, but not in COX-$1^{-/-}$ or wild-type mice.[32]

NSAIDs inhibit endogenous prostaglandin synthesis, which plays a role in controlling neoplastic and non-neoplastic cell proliferation and immune functions. Treatment of wild-type C57BL/6 mice bearing Lewis lung cancer (LLC) tumors with a selective COX-2 inhibitor reduced tumor growth. Tumors grown in COX-$2^{-/-}$ mice had lower vascular densities than those grown in wild-type mice.[32] In addition, several independent lines of research have provided support for the link between NSAID use and cancer. First, individuals who took NSAIDs regularly had 40–50% lower mortality rates from colorectal and esophageal cancers than individuals who did not use these drugs regularly.[4,6,7,9,10] Second, patients with FAP who took sulindac had significant reductions in adenoma size and number.[33] Third, several NSAIDs, including COX-2 inhibitors, have shown chemopreventive effects in animal models of carcinogenesis by causing a reduction in the frequency and number of premalignant and malignant lesions.[8] Fourth, recent studies found that an elevated level of COX-2 expression is associated with poor prognosis in cancer patients.[34,35]

Taken altogether, these findings indicate that COX-2 contributes to tumorigenesis and the malignant phenotype of tumor cells through several mechanisms by which COX-2 can (i) increase production of prostaglandins, (ii) convert procarcinogens to carcinogens, (iii) inhibit apoptosis, (iv) promote angiogenesis, (v) increase invasiveness of cancer cells, and (vi) modulate inflammation and immunoresponse. A detailed review of the contribution of COX-2 to carcinogenesis was published by Dempke *et al*.[36]

NSAIDs and COX inhibition

In the 1980s, a number of studies focused on the effects of the prostaglandins, especially PGE_2, on carcinogenesis and tumor promotion, and their inhibition by NSAIDs.[18,19,31,37] Indeed, NSAIDs can induce various cancer cell lines to undergo apoptosis.[23,38–40] Our own study showed that the COX-2 inhibitor NS398 decreased cell viability in two COX-2-positive esophageal cancer cell lines but not in a COX-2-negative cell line.[38] DNA fragmentation and terminal deoxynucleotidyl transferase-mediated dUTP end-labeling assays demonstrated that NS398 induced these COX-2-positive cells to undergo apoptosis. The percentage of apoptosis induced by NS398 was associated with the level of COX-2

expression, and the effect was inhibited by the caspase inhibitor and PGE_2. Further investigation showed that the cytochrome *c* pathway was responsible for NS398-induced apoptosis, i.e. cytochrome *c* was released from mitochondria, caspase-9 and caspase-3 were activated, and finally poly(ADP-ribose)polymerase (PARP) was cleaved.[38] This effect has been noted in a large number of different cancer cell lines, including cancers of the colon, lung, prostate, head and neck, breast, and pancreas (see following sections).

Other studies, however, discovered that NSAIDs, including COX-2 inhibitors, have COX-independent effects in cancers.[41,42] The two major arguments supporting the COX-independent effects of NSAIDs are that the dose of NSAIDs used was usually much higher than that needed to inhibit COX-2 enzymatic activity and NSAIDs are effective in cancer cells that do not express COX-2.[43] These reports demonstrated that induction of apoptosis by NSAIDs might be through 15-lipoxygenase-1,[44] arachidonic acid,[45] PPAR-δ,[46] AP-1[42,47] or NF-κB.[42,48] For example, Cao *et al*. found that exogenous arachidonic acid can cause apoptosis in colon cancer and other cancer cell lines.[45] The inhibition of COX-2 by NS398 should result in the accumulation of arachidonic acid in cancer cells and therefore trigger apoptosis. Furthermore, expression of 15-lipoxygenase-1, which is usually reduced in colorectal cancer, was induced by NS398 in colon cancer cells; this may have led to an increase in its product, 13-*S*-hydroxyoctadecadienoic acid, which can induce colon cancer cells to undergo apoptosis.[44] The adenomatous polyposis coli (APC) protein can inhibit PPAR-δ in colorectal cells and mutation of the APC gene is a major cause of colorectal carcinogenesis.[46] NSAIDs can inhibit PPAR-δ and may thereby further inhibit colorectal carcinogenesis.[46] Finally, NSAIDs have also been found to inhibit NF-κB activation and possess anti-AP-1 activity.[47,48]

Therefore, the mechanisms by which NSAIDs induce cancer cells to apoptosis can be COX-2 dependent or independent; several possible mechanisms may mediate NSAIDs' action on the induction of apoptosis and the prevention of human cancers. Whether PGE_2 is required as an autocrine factor for tumor cell survival needs further investigation.

COX-2 selective inhibitors in cancer treatment and prevention

COX-selective and -non-selective NSAIDs have been intensively evaluated for their ability to treat and prevent cancers in recent years, but the COX-2-selective inhibitors have become rather popular because they have fewer side effects than non-selective COX inhibitors. Clinically, the COX-2-selective inhibitors have been used in combination with other anti-cancer drugs or irradiation to treat solid tumors. The following summarizes their use in treatment and prevention of different cancers, with special attention to cancer prevention.

Colorectal cancer

Attention first came to the role of NSAIDs in cancer prevention and therapy from the findings of research on colorectal cancer, although aspirin and other non-selective COX inhibitors had been evaluated in animal models for their ability to prevent carcinogenesis or tumor promotion in various organ sites in the 1970s and 1980s.[18,19,49,50] NSAIDs can inhibit colorectal carcinogenesis, reducing the frequency and number of premalignant and malignant lesions in animals[5,51–56] and in humans.[8,33] After these findings, a large number of studies were performed to evaluate the activity of NSAIDs on cancer therapy and prevention, and to investigate their mechanisms of action. The overexpression of COX-2 in intestinal epithelial cells causes phenotypic changes that may enhance the tumorigenic or metastatic potential of colorectal cancer cells.[57] Oshima *et al*. demonstrated that the COX-2-null mutation dramatically reduced the number and size of intestinal polyps in mice, and that a novel COX-2 inhibitor was more effective than sulindac in reducing the number of polyps in Apc d716 mice.[55] However, several other studies have shown no correlation between COX-2 expression and apoptosis induced by NSAIDs.[42,58,59] Piazza *et al*. reported that the apoptosis induced by the NSAID sulindac metabolites in colorectal cancer cells was independent of COX inhibition or p53 induction.[58] Elder *et al*. demonstrated that there is no correlation between the sensitivity of colon cancer cell lines to NS398 and COX-2 expression or the addition of PGE_2 and the induction of apoptosis in those cell lines, although COX-2 protein was expressed in three adenoma cell lines, evidence that COX-2 is expressed at an early stage of human colorectal cancers.[60] Our recent data showed that colon cancer cells with COX-2 expression were more sensitive to treatment with NS398 than colon cancer cells not expressing COX-2; however, the dose used was higher than the dose needed for inhibition of COX-2, indicating that COX-2-independent actions of NS398 do exist.[39]

Esophageal cancer

Epidemiological and experimental studies have provided support for the link between NSAID use and decreased esophageal cancer incidence. Individuals who took NSAIDs regularly had a 40–50% lower rate of mortality from esophageal cancer than individuals who did not use these drugs.[7,9,10,61] Furthermore, several NSAIDs have shown chemopreventive effects in animal models of esophageal carcinogenesis by causing a reduction in the frequency and number of premalignant and malignant lesions.[7,62,63] As in colorectal cancer, COX-2 is overexpressed in tissue specimens and cell lines of esophageal cancer.[23,64–68] Tobacco carcinogen benzo[*a*]pyrene diol epoxide (BPDE) and tumor promoting bile acids can induce COX-2 expression in esophageal cancer cells.[23–25]

To evaluate the effects of NSAIDs on esophageal cancer cell lines and their mechanisms of action we examined for the first time the effect of aspirin on growth and apoptosis in 10 esophageal cancer cell lines as well as the expression and modulation of its target enzymes (i.e. the COXs) and their product PGE_2. Growth inhibition of these cells by aspirin was dose- and time-dependent, and associated with induction of apoptosis.[23] COX-1 and -2 were expressed in seven of the 10 cell lines. Bile acids were able to induce COX-2 expression in six of eight cell lines tested, which was correlated with PGE_2 production, and aspirin could inhibit COX-2 enzymatic activity even after bile acid stimulation, but was unable to change the COX-2 protein level in these cell lines. Aspirin was found to down-regulate *bcl-2* in the two cell lines tested.

We also examined the effects of COX-2 inhibitor NS398 on esophageal cancer cells.[38] NS398 can induce esophageal cancer cells to undergo apoptosis, and the percentage of apoptosis induced by NS398 was associated with the level of COX-2 expression. Further investigation showed that the cytochrome *c* pathway was responsible for NS398-induced apoptosis in these cancers just as it was in colorectal cancers. The effects of NS398 in these cell lines were inhibited by the caspase inhibitor and PGE_2.[38] Another recent report on the effects of NS398 on esophageal adenocarcinoma cells demonstrated that treatment with COX-1-selective concentrations of flurbiprofen did not affect cell growth in any of the three tumor cell lines tested.[69] In contrast, treatment with COX-2-selective concentrations of NS398 significantly suppressed cell growth and increased apoptosis in the cell lines that expressed COX-2, but not in the cell line that did not express COX-2, which supports our findings. Altogether, these studies support the use of COX-2 inhibitors in esophageal cancer prevention and therapy.

Lung cancer

As early as 1984, Young and Knies demonstrated that growth of LLC cells was correlated with an increase in PGE level in the culture medium, which can be prevented by adding indomethacin.[70] As the tumors developed in mice xenografts, systemic levels of PGE increased; treatment with exogenous PGE_2 resulted in an increase in the frequency of tumor establishment, while oral administration of indomethacin to LLC-implanted mice resulted in a reduced rate of tumor establishment, growth and metastasis.[70] More recent animal experiments demonstrated that COX-2 inhibitors can reduce tumor formation, progression and metastasis, and inhibit angiogenesis in different lung carcinogenesis models.[12,71,72] For example, Rioux *et al*. reported that, in A/J mice, NS398 inhibited by 34% multiplicity of lung tumor induced by administration of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the drinking water; NS398 also returned plasma PGE_2 to basal levels (i.e. levels in untreated mice).[12] Treatment with aspirin had the same effects.[12]

Several reports indicated that COX-2 expression is increased in lung cancer tissues[73–75] and its expression is correlated with patient prognosis.[34,35] COX-2 inhibitors alone or in combination with other anticancer drugs can decrease lung cancer cell growth and induce apoptosis.[76] A limited number of epidemiological studies supported the suggestion that regular use of NSAIDs could reduce lung cancer incidence.[13] Together, all of these lines of evidence prompted a clinical trial in our Institute to test the ability of celecoxib to prevent lung cancer in former and current smokers.

Breast cancer

Despite convincing evidence from animal experiments, the findings of epidemiological studies linking the use of NSAIDs with lower risk of breast cancer have been equivocal. For example, Coogan *et al*., in a hospital-based case-controlled study of 6558 patients, demonstrated a small reduction of breast cancer incidence in regular NSAID users.[77] Other studies by Harris *et al*. also found a significant

reduction of breast cancer incidence in NSAIDs users.[78,79] They conducted a prospective cohort study among 32505 women in central Ohio, USA. After 5 years of follow-up, a total of 393 cases of breast cancer have been detected. The annual incidence of breast cancer per 100 000 women varied inversely with increasing intake of NSAIDs, declining from 323 among non-users to 183 among heavy users. Breast cancer rates decreased by about 50% with regular ibuprofen intake and by about 40% with regular aspirin intake.[78] However, another study by Egan *et al*. concluded that regular aspirin use did not reduce the risk of breast cancer.[80] They studied a population of 89 528 female registered nurses who reported no history of breast or other cancers (excluding non-melanoma skin cancer) for 12 years. Breast cancer cases were identified through questionnaire responses and confirmed through examination of medical records, which yielded 2414 cases of invasive breast cancer (2303 cases confirmed with medical records and 111 cases for which no records were obtained). Regular aspirin use was unrelated to breast cancer incidence during the succeeding 12-year period. The corresponding risk estimate for consistent regular aspirin use during the period from 1980 through 1988 was 1.01. The risks were similar for heavy aspirin use: the multivariate relative risks (RRs) in 1980 and in 1980 through 1988 were 1.05 and 1.09, respectively.[80]

On other hand, animal experiments showed very promising data.[81–83] A Japanese group investigated the ability of nimesulide, a selective COX-2 inhibitor, to prevent carcinogenesis in the mammary glands of rats that was induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).[81] The COX-2 protein was overexpressed in epithelial cancer cells and stroma cells of the PhIP-induced mammary carcinomas but weakly or not at all in normal mammary gland cells. The development of mammary carcinomas was clearly suppressed by administration of nimesulide. The carcinoma incidence was 51% in the group that received nimesulide and 71% in the control diet group. The size of carcinomas that did develop was also clearly smaller in the group that received the NSAID.[81] Again, Harris *et al*. used a non-selective COX inhibitor ibuprofen and a COX-2 inhibitor celecoxib to prevent breast cancers in another animal model, in which mammary carcinogenesis was induced by 7,12-dimethyl-benz[*a*]anthracene in female Sprague-Dawley rats.[83] Their data showed that dietary administration of celecoxib (1500 p.p.m.) produced striking reductions in the incidence, multiplicity and volume of breast tumors relative to the control group. Ibuprofen also produced significant effects, but of lesser magnitude. The same group has also reported that celecoxib significantly decreased the size of the established mammary tumors in rats.[84] At the end of the 6-week treatment, average tumor volume was 1.45 and 0.13 cm^3 in the control and celecoxib-treated rats, respectively.[84] Another study showed that a COX-2 inhibitor, NS398, retarded tumor progression in a murine mammary tumor model by inhibiting tumor cell migration, invasiveness and tumor-induced angiogenesis.[82]

In humans, the COX-2 protein is upregulated in 56% of breast cancers, which was significantly different from its negligible expression in distant non-neoplastic epithelium.[85] Further clinical trials are needed to determine whether COX-2 inhibition represents a useful approach to preventing or treating human breast cancer.[86]

Bladder cancer

Prevention of bladder cancer with NSAIDs has been traced back to the early 1980s. Studies at that time demonstrated that aspirin can inhibit *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced lesions of the urinary bladder and reduce PGE_2 production in the rat bladder.[87–90] In the 1990s, the COX-2 inhibitor piroxicam was shown to inhibit induction of transitional cell carcinoma in mouse urinary bladder by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. In mice receiving a diet containing 15 mg piroxicam/kg, tumor incidence was reduced 82% compared with the carcinogen controls.[91] In another study, 34 dogs with histopathologically confirmed, measurable, non-resectable transitional cell carcinoma of the urinary bladder were treated with piroxicam; tumor responses comprised complete remissions in two dogs, partial remissions in four, stable disease in 18 and progressive disease in 10. The median survival duration of all the dogs was 181 days. The authors concluded that anti-tumor activity, which was not likely the result of a direct cytotoxic effect, was observed in dogs with transitional cell carcinoma of the bladder treated with piroxicam.[92]

Another more recent study demonstrated that celecoxib effectively inhibited tumor growth and prolonged survival in the mouse model of urinary bladder cancer. Celecoxib also profoundly inhibited development of urinary bladder cancers in the rat model even when administered following the last dose of the carcinogen *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine.[15] In humans, COX-2 expression was high in most invasive transitional cell carcinomas of

the urinary bladder and in carcinoma *in situ* and much less in normal urinary bladder samples.[93–96] A population-based case-controlled epidemiological study demonstrated that NSAID users had a reduced overall risk of bladder cancer.[14] The study was conducted in Los Angeles, California, and involved 1514 incident bladder cancer cases and an equal number of controls. Regular use of analgesics was not associated with an increased risk of bladder cancer in either men or women; in fact, compared with non-users or irregular users, regular analgesic users were at a decreased risk of bladder cancer overall.[14] To further evaluate the effects of COX-2 inhibitors in preventing bladder cancer, a clinical trial of celecoxib is now in progress in our Institution.

Prostate cancer

In laboratories, COX-2 inhibitors have been shown to induce human prostate cancer cell lines to undergo apoptosis both *in vitro* and *in vivo*.[97–99] The *in vivo* results indicated that COX-2 inhibitors decreased tumor microvessel density and angiogenesis, and prevented hypoxic upregulation of a potent angiogenic factor, vascular endothelial growth factor.[97,99] COX-2 is elevated in prostate cancer and prostate intraepithelial neoplasia.[100–104]

However, epidemiological studies of NSAID use elicited different outcomes in prostate cancer. A study by Nelson *et al*. showed that regular daily use of over-the-counter NSAIDs (ibuprofen or aspirin) was associated with a 66% reduction in prostate cancer risk in a case-controlled study of 417 patients with prostate cancer and 420 group-matched control subjects.[105] However, another case-controlled study using the general practice research database of 12 174 cancer cases and 34 934 controls demonstrated that the risks of prostate cancer were increased among patients who had received at least seven prescriptions of NSAIDs.[61] However, protective effects were seen against cancers of the esophagus, stomach, colon and rectum with dose-related trends.[61] Altogether, the effects of COX-2 inhibitors in prevention of prostate cancer need further investigation.[97]

Skin cancer

Skin cancer is the single most frequently diagnosed cancer in the Western population. Of the two most common forms, squamous cell and basal cell carcinoma, squamous cell carcinoma (SCC) of the skin is clinically more aggressive, accounts for most skin cancer deaths, and is thought to be caused by significant and cumulative sun exposure in occupational and recreational activities.[106] Ultraviolet (UV) irradiation from sunlight is thought to be the major cause of human skin cancer.[47] Recent studies demonstrated that COX-2 is usually elevated in skin cancers, and that UV irradiation can markedly increase COX-2 expression in benign papillomas and in SCCs.[47,107–112] The COX-2 inhibitor celecoxib effectively reduced UV light-induced skin cancer formation in hairless mice[113] and also was shown to reduce many parameters of UVB-mediated inflammation, including edema, dermal neutrophil infiltration and activation, PGE_2 levels, and the formation of sunburn cells.[113] In another study reported by Fisher *et al*., SKH:HR-1-hrBr hairless mice with UV-induced skin carcinogenesis fed 150 or 500 p.p.m. celecoxib showed a dose-dependent reduction (60 and 89%, respectively) in tumor yield.[114] Indomethacin (4 p.p.m.) reduced tumor yield by 78%. However, although both acute and chronic UV exposure increased cell proliferation and edema, neither NSAID reduced these parameters.[114] These dramatic protective effects of celecoxib suggest that specific COX-2 inhibitors may offer a way to safely reduce the risk of skin cancer in humans.

Combination of COX-2 inhibitors and other anti-cancer agents

The first major combination anti-cancer therapy of COX-2 with other agents was reported by Torrance *et al*., in which colorectal adenomas in mice were prevented by using the combination of COX-2 and an inhibitor of the epidermal growth factor (EGF), and the synergistic effect may have been due to the convergence of these two signaling pathways.[115] More recently, Mann *et al*. evaluated whether simultaneous targeting of COX-2 and HER-2/*neu* pathways inhibits colorectal carcinoma growth.[116] These studies may have brought us to a new era for combination of NSAIDs with other drugs.

Our recent data have demonstrated that NS398 can induce colon and esophageal cancer cells to undergo apoptosis through the cytochrome *c*-dependent pathway.[38,39] These findings suggest that potential therapeutic regimens combing NSAIDs with other apoptosis-inducing drugs whose mechanisms of action are independent of cytochrome *c* pathways (e.g. ceramide) may be worthwhile pursuing.

Other reports,[21,22] including ours,[117] showed that retinoids suppressed both basal levels and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or EGF-mediated induction of COX-2 protein and synthesis of PGE_2. Treatment with BPDE and tumor-promoting bile acids resulted in significant increases in COX-2 enzyme in different cancer cell lines, and all-*trans* retinoic acid can markedly suppress COX-2 induction and production of PGE_2.[24,25] Retinoids also suppressed induction of COX-2 mRNA in TPA-treated cancer cells.[21] Our recent study demonstrated that the anti-cancer effect of retinoic acid receptor (RAR)-β may be related to its ability to suppress COX-2 expression and supported the suggestion that loss of RAR-β expression may contribute to human carcinogenesis.[117] A possible mechanism for this is that RAR-β mRNA is frequently lost during cancer development in various organs[118] and cancer cells that do not express RAR-β are resistant to retinoic acid, especially in lung and esophageal cancers.[119,120] Both BPDE and bile acids can dramatically reduce RAR-β expression in esophageal cancer cells, and transfection of RAR-β decreased cell growth and colony formation and induced apoptosis in different cancer cells.[24,117] Our recent data demonstrated that induction of RAR-β decreased COX-2 expression in RAR-β-transfected esophageal cancer cells, whereas transfection with antisense RAR-β increased COX-2 expression. The inhibitory effect of RAR-β on COX-2 expression was further enhanced in the presence of retinoic acid, which was blocked by an RAR antagonist. Meanwhile, retinoic acid blocked bile acid-induced COX-2 expression and PGE_2 production only in the RAR-β-positive cells.[117] Taken together, these findings suggest that combining COX-2 inhibitors with retinoic acids may achieve synergistic or additive effects.

The effects of combinations of COX-2 inhibitors and radiation or anti-cancer drugs on different cancer cells were evaluated recently by different research groups.[121–123] These *in vitro* and *in vivo* data suggest that selective inhibition of COX-2 combined with radiation or anti-cancer drugs has potential as a cancer treatment.

Future directions

Both NSAIDs and COX-2-selective inhibitors are very effective against various solid cancers both *in vitro* and *in vivo* despite their different mechanisms of action. Therefore, a number of clinical trials of COX-2 inhibitors have been initiated in patients with increased risks of developing cancers such as colon, lung, bladder and esophageal tumors. The data from such studies will contribute significantly to our understanding of both carcinogenesis and COX-2 inhibition in the prevention of human cancers.

Moreover, the combination of COX-2 inhibitors with other chemopreventive or chemotherapeutic drugs is warranted. In the near future, the combination of COX-2 inhibitors with other drugs such as retinoids and curcumin may be another choice for cancer prevention or treatment, one that could achieve synergistic or additive effects because COX-2 enzyme is elevated in a large number of cancer tissues, and the combination can reduce both COX-2 protein expression and its enzymatic activity. At the same time, this would allow the concentrations of both drugs, and thus their side effects, to be dramatically decreased. The combination of COX-2 inhibitors with other chemotherapy drugs or irradiation has been evaluated because COX-2 inhibitors induce various cancer cell lines to undergo apoptosis through the cytochrome *c* pathway, suggesting that their combination with other apoptosis-inducing drugs (e.g. ceramide) should have synergistic or additive effects. Finally, many questions about the COX-2 inhibitors, especially their mechanisms of action, remain unresolved and need further investigation despite rapid progression in this field during last several years.

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