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REVIEW ARTICLE

Cyclooxygenase-2 and Epidermal Growth Factor Receptor: Pharmacologic Targets for Chemoprevention

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ABSTRACT

Understanding the mechanisms underlying carcinogenesis provides insights that are necessary for the development of therapeutic strategies to prevent cancer. Chemoprevention, the use of drugs or natural substances to inhibit carcinogenesis, is a rapidly evolving aspect of cancer research. Evidence is presented that cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) are potential pharmacologic targets to prevent cancer. In this paper, we review key data implicating a causal relationship between COX-2, EGFR, and carcinogenesis and possible mechanisms of action. We discuss evidence of crosstalk between COX-2 and EGFR in order to strengthen the rationale for combination chemoprevention, and review plans for a clinical trial that will evaluate the concept of combination chemoprevention targeting COX-2 and EGFR.

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INTRODUCTION

Global statistics on cancer indicate that in the year 2000 there were 10.1 million new cases, 6.2 million deaths, and 22 million people living with the disease.[1] These statistics underscore the need to identify new and improved approaches to prevent cancer. Chemoprevention represents one of several promising strategies to reduce the cancer burden. Extensive efforts are underway to develop targeted therapies that will inhibit tumorigenesis. In this regard, cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) represent two of the more promising pharmacologic targets that have been identified. Crosstalk exists between COX-2 and EGFR.[2] In preclinical studies, combining an inhibitor of COX-2 with an inhibitor of EGFR tyrosine kinase was more effective than either agent alone in suppressing tumor formation.[3] Here we focus on the rationale for targeting COX-2 and EGFR as a strategy to prevent or delay the development of human malignancies.

PROSTAGLANDIN BIOSYNTHESIS

COX enzymes catalyze the synthesis of prostaglandins (PGs) from arachidonic acid (Fig 1). The first step in PG synthesis is hydrolysis of phospholipids to produce free arachidonic acid. This reaction is catalyzed by phospholipase A_2. Next, COX catalyzes a reaction in which molecular oxygen is inserted into arachidonic acid to form an unstable intermediate, PGG_2, which is rapidly converted to PGH_2. Specific isomerases then convert PGH_2 to several PGs and thromboxane A_2 (TxA_2).

There are two isoforms of COX: COX-1 and COX-2. These two enzymes differ in many respects.[4,5] COX-1 is expressed constitutively in most tissues and appears to be responsible for the production of PGs that control normal physiologic functions including maintenance of the gastric mucosa, regulation of renal blood flow and platelet aggregation. In contrast, COX-2 is not detected in most normal tissues. However, it is rapidly induced by both inflammatory

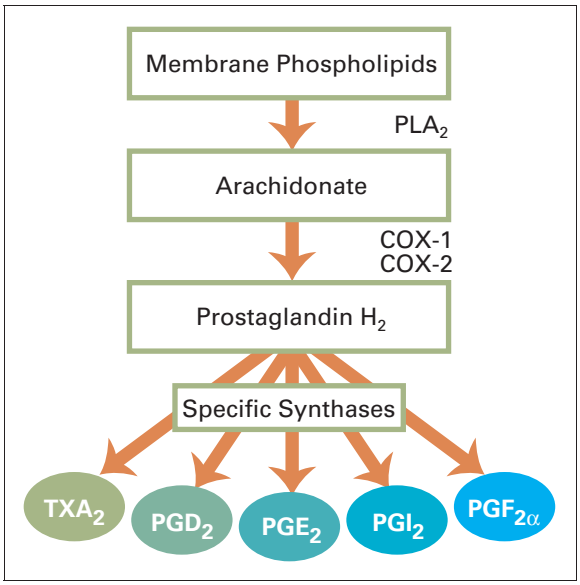

Fig 1. Arachidonic acid metabolism. Arachidonic acid is released from membrane phospholipids by phopholipase A_2 (PLA_2). It is then metabolized by cyclooxygenases (COX-1, COX-2) to prostaglandin H_2 (PGH_2). PGH_2 is converted to a variety of eicosanoids by specific synthases.

and mitogenic stimuli resulting in increased PG synthesis in neoplastic and inflamed tissues.[5,6] COX-2 can be selectively inhibited even though the active sites of COX-2 and COX-1 have similar structures. A substitution of isoleucine in COX-1 with valine in the nonsteroidal anti-inflammatory drug (NSAID) binding site of COX-2 creates a void volume located to the side of the central active site channel in COX-2.[7] Compounds synthesized to bind in this additional space inhibit COX-2, but not COX-1. In contrast to conventional NSAIDs that are dual inhibitors of COX-1/COX-2, selective COX-2 inhibitors do not suppress platelet function and thereby increase the risk of a bleeding complication.[8]

REGULATION OF COX-2 EXPRESSION

Increased amounts of COX-2 are commonly found in both premalignant and malignant tissues (Table 1).[9-27] Overexpression of COX-2 occurs because of deregulated transcriptional and post-transcriptional control. Growth factors, oncogenes, cytokines, and tumor promoters stimulate *COX-2* transcription via protein kinase C (PKC) and Ras-mediated signaling (Fig 2).[2,5,6,28-31] For example, increased amounts of COX-2 have been observed in breast cancers that overexpress HER-2/*neu* because of enhanced Ras signaling (Fig 2).[29] Depending on the cell type and stimulus, different transcription factors including activator protein-1, NF-IL6, NF-κB, NFAT and PEA3 can activate *COX-2* transcription.[5,28,29,32,33] Although *COX-2* transcription can be stimulated by many factors, much less is known about negative effectors. Wild-type, but not mutant p53, can inhibit *COX-2* transcription in vitro.[34] Consistent with this finding, elevated levels of COX-2 have been found in cancers of the stomach, esophagus, lung, and breast that express mutant rather than wild-type p53.[35,36] Like p53, *APC* tumor suppressor gene status may also impact COX-2 expression.[37] Taken together, these findings suggest that the balance between activation of oncogenes and inactivation of tumor suppressor genes modulates the expression of COX-2 in tumors.

Post-transcriptional mechanisms also appear to play an important role in regulating amounts of COX-2 in tumors. The 3′-untranslated region (UTR) of COX-2 mRNA contains a series of AU-rich elements (AREs) that affect both mRNA decay and protein translation (Fig 2).[38] Trans-acting ARE binding factors form complexes with the COX-2 3′-UTR and regulate both COX-2 mRNA stability and translation. Enhanced binding of HuR, an RNA binding protein, to the AU-enriched region of the COX-2 3′-UTR contributes to the increase in message stability found in colon cancer (Fig 2).[39] Other proteins (eg, tristetraprolin, AUF1) that bind to the 3′-UTR can enhance mRNA degradation.[40] Overexpression of COX-2 may also reflect deregulated translation. Recently, TIA-1, an ARE binding protein, was found to function as a translational silencer. Deficient TIA-1 mRNA binding was found in colon cancer cells that overexpressed

Table 1. COX-2 Is Overexpressed in Premalignant and Malignant Tissues

Organ	Premalignancy	Malignancy
Head and neck	Leukoplakia	Squamous cell carcinoma
Esophagus	Barrett's esophagus	Adenocarcinoma; squamous cell carcinoma
Stomach	Metaplasia	Adenocarcinoma
Colon	Adenoma	Adenocarcinoma
Liver	Chronic hepatitis	Hepatocellular carcinoma
Biliary System	Bile duct hyperplasia	Cholangiocarcinoma; adenocarcinoma of gall bladder
Pancreas	Pancreatic intraepithelial neoplasia	Adenocarcinoma
Breast	Ductal carcinoma-in-situ	Adenocarcinoma
Lung	Atypical adenomatous hyperplasia	Adenocarcinoma; squamous cell carcinoma
Bladder	Dysplasia	Transitional cell carcinoma; squamous cell carcinoma
Gynecologic	Cervical intraepithelial neoplasia	Squamous cell carcinoma or adenocarcinoma of cervix; endometrial carcinoma
Penis	Penile intraepithelial neoplasia	Squamous cell carcinoma
Skin	Actinic keratoses	Squamous cell carcinoma

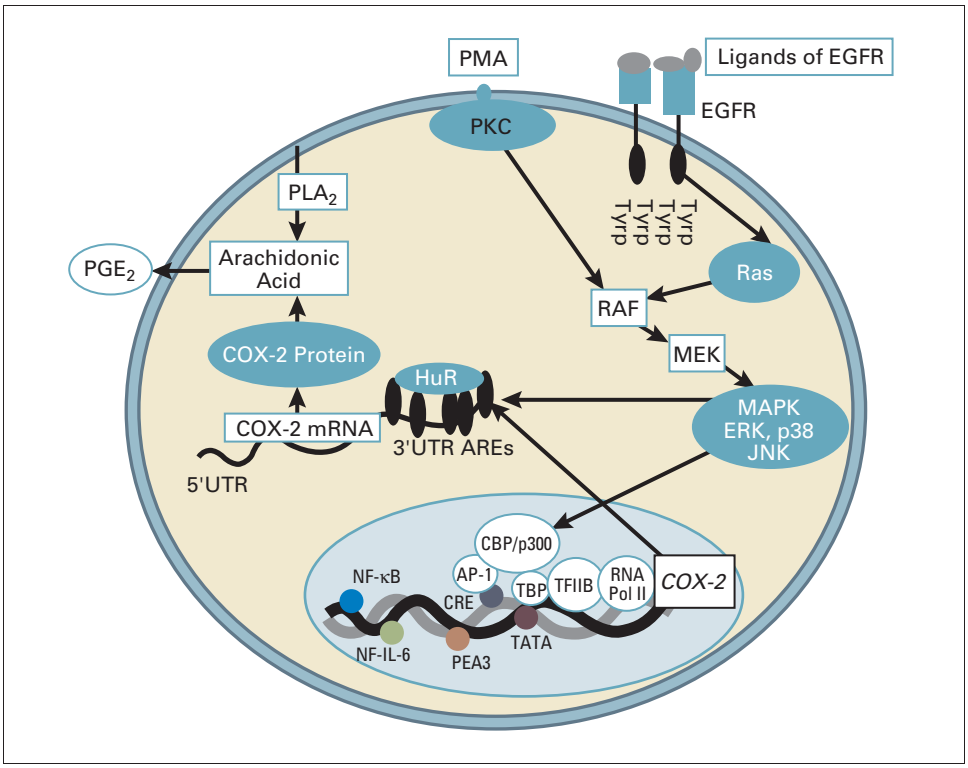

Fig 2. Regulation of cyclooxygenase-2 (COX-2) expression in cancers. COX-2 is induced by a variety of stimuli including oncogenes, growth factors and tumor promoters (phorbol esters, PMA). Stimulation of Ras or protein kinase C (PKC) signaling enhances mitogen-activated protein kinase (MAPK) activity that results, in turn, in increased *COX-2* transcription. A variety of transcription factors including AP-1 and PEA3 mediate the induction of COX-2. Levels of COX-2 can also be affected by post-transcriptional mechanisms. The 3′-untranslated region (3′-UTR) of COX-2 mRNA contains a series of AU-enriched elements (ARE) that regulate message stability. Augmented binding of HuR, an RNA binding protein, to the AREs of the COX-2 3′-UTR explains, in part, the observed increase in COX-2 message stability in some tumors.

COX-2 protein.[41] Collectively, these findings suggest that changes in the relative abundance or binding activity of these functionally distinct ARE-binding proteins are likely to modulate amounts of COX-2 in tumors.

PROSTAGLANDIN RECEPTORS, SIGNALING, AND CARCINOGENESIS

Overexpression of COX-2 leads to increased amounts of prostanoids in tumors. Prostanoids affect numerous mechanisms that have been implicated in carcinogenesis. For example, PGE_2 can stimulate cell proliferation and motility while inhibiting immune surveillance and apoptosis.[42-50] Importantly, PGE_2 can also induce angiogenesis, at least in part, by stimulating the production of proangiogenic factors including vascular endothelial growth factor.[51,52] These important mechanisms linking COX-2-derived PGs to carcinogenesis have been reviewed recently.[4,53,54] Defining the downstream signaling mechanisms by which prostanoids stimulate carcinogenesis is an active area of investigation. Prostanoids (PGE_2, $PGF_{2\alpha}$, PGD_2, TxA_2 and PGI_2) mediate their biologic actions by binding to G protein–coupled receptors that contain seven transmembrane domains. Multiple prostanoid receptors have been cloned and defined pharmacologically, including four subtypes of the EP (PGE) receptor (EP_1, EP_2, EP_3, EP_4), the FP receptor (PGF receptor), the DP receptor (PGD receptor), the IP receptor (PGI receptor) and the TP receptor (Tx receptor). PGE_2 is the most abundant prostanoid detected in most epithelial malignancies. Because it can stimulate tumor growth, numerous studies have attempted to define the link between PGE_2, EP receptors, and carcinogenesis.

EP receptors play an important role in the development and growth of tumors. The availability of EP receptor knockout mice has facilitated studies of tumor growth, immune function, and angiogenesis. PGE_2 promotes the formation of colorectal carcinogenesis through activation of EP receptors. In support of this idea, the induction of aberrant crypt foci by azoxymethane, a colon carcinogen, was reduced in $EP_1^{-/-}$ and $EP_4^{-/-}$–receptor mice.[55] In $Apc^{\Delta 716}$ mice, a murine model of familial adenomatous polyposis (FAP), homozygous deletion of the gene encoding the EP_2 receptor, caused a significant decrease in the number and size of intestinal polyps through suppression of angiogenesis.[56] Inhibition of angiogenesis was due, at least in part, to a decrease in levels of vascular endothelial growth factor. The importance of host stromal PGE_2-EP_3 signaling was highlighted in a xenograft study that found a marked reduction in tumor-associated angiogenesis in $EP_3^{-/-}$ mice.[57] PGE_2 also exerts potent immunosuppressive effects by modulating dendritic cell function and causing an imbalance between type 1 and type 2 cytokines.[58] An important role has been established for the EP_2 receptor in PGE_2-mediated suppression of dendritic cell differentiation and function and for reduced antitumor cellular immune responses in vivo.[59]

Complementary in vitro studies have provided significant insights into procarcinogenic signaling mechanisms that are activated by PGE_2. For example, stimulation of either EP_2 or EP_4 activates TCF-β-catenin–mediated transcription that leads, in turn, to increased expression of a variety of genes (eg, *cyclin D1* and *c-myc*) that have been implicated in carcinogenesis (Fig 3).[60] PGE_2 also has organ site-specific effects. Estrogen drives the growth of hormone-dependent breast cancer. The final step in the synthesis of estrogen is catalyzed by aromatase, the product of the *CYP19* gene. Binding of PGE_2 to EP receptors stimulates adenylyl cyclase activity and enhances production of cyclic adenosine monophosphate (cAMP), which in turn induces the transcription of the gene encoding aromatase via CREB.[61] Consequently, estrogen biosynthesis is increased, which leads to enhanced proliferation of tumor cells. In addition to PGE_2, other prostanoids including TxA_2 and PGI_2 impact carcinogenesis, but less is known about the downstream signaling mechanisms.[4,62]

PRECLINICAL EVIDENCE THAT TARGETING COX-2 INHIBITS CARCINOGENESIS

As mentioned above, COX-2-derived prostanoids have numerous procarcinogenic effects. It is reasonable to postulate, therefore, that inhibiting COX-2 activity will suppress carcinogenesis. To investigate this possibility, numerous studies have been carried out in experimental animals. The most specific data supporting a cause-and-effect relationship between COX-2 and carcinogenesis come from genetic studies. Multiparous female transgenic mice engineered to overexpress human *COX-2* in mammary glands developed metastatic tumors.[63] In another related study, transgenic mice that overexpressed COX-2 in basal keratinocytes developed epidermal hyperplasia and dysplasia.[64] This implies a causal link between expression of COX-2 and the development of premalignant lesions of the skin. Consistent with the overexpression data, a marked decrease in the development of both intestinal tumors and skin papillomas was observed in COX-$2^{-/-}$ mice.[65,66] The importance of arachidonic acid metabolism in tumorigenesis is underscored by the observation that knocking out the *COX-1* gene also protected against the formation of intestinal and skin tumors.[66]

In addition to genetic evidence, numerous pharmacologic studies suggest that COX-2 is a therapeutic target. Treatment with selective inhibitors of COX-2 reduced the formation of tongue, esophageal, intestinal, breast, skin, lung, and bladder tumors in experimental animals.[67-76] Taken together, the results of both genetic and pharmacologic studies suggest that COX-2 warrants further investigation as a molecular target for the prevention of human cancer.

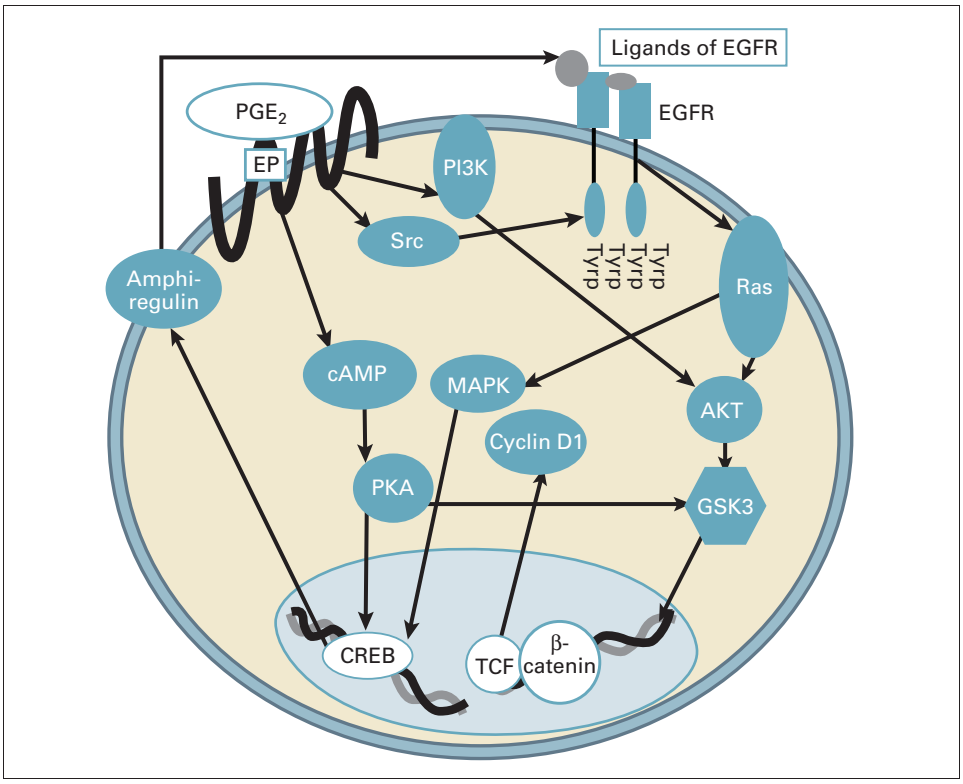

Fig 3. Prostaglandin E_2 (PGE_2) activates signal transduction pathways that have been implicated in carcinogenesis. PGE_2 activates cellular signaling in an EP-receptor–dependent manner. For example, PGE_2-mediated activation of EP_2 and EP_4 receptors leads to enhanced adenylate cyclase activity and cyclic adenosine monophosphate (cAMP) production. cAMP, in turn, activates protein kinase A (PKA) -CREB dependent expression of genes including amphiregulin. Amphiregulin, a ligand of epidermal growth factor receptor (EGFR), stimulates EGFR-Ras-mitogen-activated protein kinase (MAPK) signaling. Additionally, activation of EP_2 or EP_4 stimulates TCF-β-catenin-mediated transcription of genes including cyclin D1. Both the cAMP/PKA and phosphatidylinositol 3-kinase (PI3K) signaling pathways have been implicated in the activation of transcription by TCF-β-catenin.

USE OF SELECTIVE COX-2 INHIBITORS TO PREVENT HUMAN CANCERS

To be useful in humans, a chemopreventive agent needs to have an acceptable risk:benefit ratio. An individual's personal risk of developing cancer will affect whether he or she is willing to undertake preventive measures and tolerate possible side effects. For example, an individual with a germline mutation in a tumor suppressor gene, such as *APC*, that predisposes to colon cancer, is much more likely to be willing to undergo preventive therapy than a person at average risk for colon cancer. In this context, it is noteworthy that selective COX-2 inhibitors cause less injury to the upper gastrointestinal tract than conventional NSAIDs.[77] The first clinical trial to evaluate the anticancer properties of a selective COX-2 inhibitor was carried out in FAP patients. This patient population was chosen because of the strength of the preclinical data and prior evidence that sulindac, a dual inhibitor of COX-1/COX-2, reduced the number of colorectal polyps in FAP patients.[78] Treatment with celecoxib 400 mg bid for 6 months led to a 28% reduction in the number of colorectal polyps ($P = .003$).[79] Based on these results, the United States Food and Drug Administration approved celecoxib as adjunctive therapy for the management of polyps in FAP patients. In a more recent study, rofecoxib, another selective COX-2 inhibitor, was also found to decrease the number and size of rectal polyps in FAP patients.[80] Because of similarities in the biology of FAP and sporadic colorectal cancer, therapeutic strategies that are effective in FAP might also be useful in patients with a history of colorectal adenomas. Adenomas are the precursors of the majority of colorectal cancers. Hence, treatments that suppress the formation of premalignant adenomas should protect against the development of colorectal cancers. Several large clinical trials are underway to assess the efficacy of selective COX-2 inhibitors in preventing sporadic colorectal adenomas.

As detailed above, selective COX-2 inhibitors protect against the formation of multiple tumor types in experimental animals. Ongoing phase II trials are building upon these preclinical findings by evaluating the potential efficacy of a selective COX-2 inhibitor in a variety of target organs. At risk cohorts include patients with oral premalignant lesions, bronchial metaplasia, Barrett's dysplasia, basal cell nevi, and actinic keratoses. Given the frequent need for surgical intervention in conditions such as Barrett's dysplasia and oral leukoplakia, developing a pharmacologic strategy to cause either regression or stabilization of disease would represent a significant clinical advance. Another study will determine whether a selective COX-2 inhibitor can delay or prevent the recurrence of superficial bladder cancer.

Clearly, significant progress has been made in establishing a link between COX-2, PGs, and carcinogenesis. As discussed above, this has provided a strong rationale for numerous clinical trials that are ongoing. In parallel, other potential pharmacologic targets such as EGFR have been identified. The link between EGFR and carcinogenesis and the potential for targeting EGFR as an approach to cancer prevention is discussed in the next section.

EGFR SIGNALING AND CANCER PREVENTION

Epidermal growth factor (EGF) was initially discovered in the early 1960s when bioassays revealed accelerated eyelid opening in animals treated with protein extracts prepared from submaxillary glands.[81] In one of his first publications describing the discovery of EGF, Cohen[81] predicted that this growth factor receptor pathway would someday have significant implications in the treatment of cancer and possibly other diseases. Over the past 40 years, a great deal of progress has been made in improving our understanding of the molecular mechanisms responsible for the biologic effects of this growth factor and EGFR (ErbB-1).[82]

The ErbB family of receptors includes EGFR, ErbB-2 (HER2), ErbB-3 (HER3) and ErbB-4 (HER4; Fig. 4). Binding of ligands, including EGF, to the ectodomain of these receptors results in the formation of homodimeric and heterodimeric complexes, which is followed rapidly by activation of the receptors' intrinsic tyrosine kinase. Phosphorylation of specific C-terminal tyrosine residues and the recruitment of specific second messengers activate a range of intracellular signaling pathways that play key roles in development, differentiation, migration, and proliferation. Clearly, the ErbB signaling pathways are very important in development because homozygous null mutations of ErbB family members results in an embryonic lethal phenotype.

Activation of ErbB receptor signaling has been linked to cancer. Mechanisms involved in activation of the ErbB receptor pathway include: (1) receptor overexpression,[83] (2) mutant receptors resulting in ligand-independent activation,[84,85] (3) autocrine activation by overproduction of ligand,[82] and (4) transactivation through other receptor systems.[86,87] Overexpression of EGFR correlates with poor prognosis in several malignancies.[83,88] Importantly, EGFR signaling induces its cognate ligands, creating autocrine loops that can amplify EGFR activity. Several major signaling pathways mediate the downstream effects of activated EGFR (Fig 4). Activation of EGFR can stimulate the Ras→Raf→MAP kinase (MAPK) pathway.[89] Elevated MAPK activity has been reported in a number of tumors when compared with corresponding nonneoplastic tissues, and correlated with EGFR and ligand expression.[90] A second EGFR-driven pathway involves phosphatidylinositol 3-kinase and Akt (Fig 4).[91,92] Activation of EGFR can also

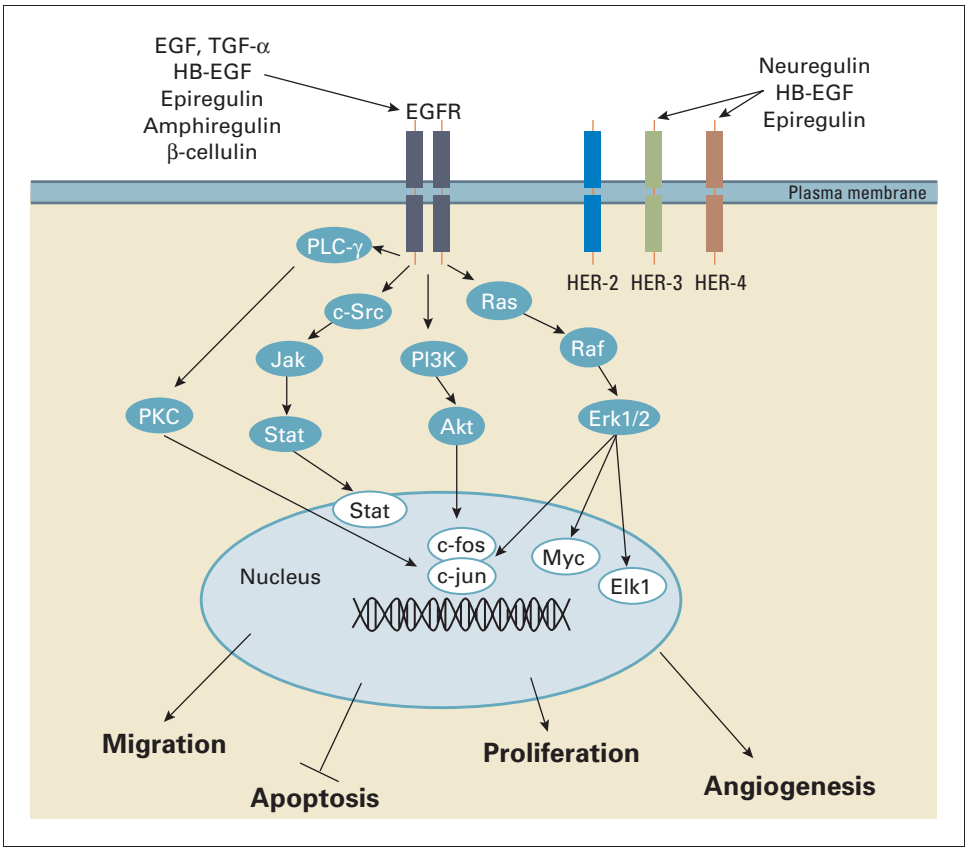

Fig 4. Epidermal growth factor receptor (EGFR) signal transduction pathways that are implicated in carcinogenesis. EGFR is a member of the ErbB tyrosine kinase receptor family, which also includes HER2, HER3, and HER4. The ErbB receptors are present in the plasma membrane and share a common structure composed of an extracellular ligand-binding domain, transmembrane segment, and an intracellular tyrosine kinase domain. ErbB receptors are activated by a variety of receptor-specific ligands including epidermal growth factor (EGF), transforming growth factor-alfa (TGF-α), amphiregulin, heparin-binding EGF (HB-EGF), epiregulin, neuregulin, and betacellulin. As a result of ligand binding, receptor dimerization, trans-autophosphorylation, and initiation of signaling occur. Several major signaling pathways mediate the downstream effects of EGFR activation. One pathway involves Ras→Raf→MAP kinase. A second pathway involves phosphatidylinositol 3-kinase (PI3K) and Akt. Activation of EGFR can also lead to enhanced signaling via Jak/Stat or protein kinase C (PKC). Many carcinogenic processes are mediated by EGFR signaling including cell survival, proliferation, angiogenesis, and invasiveness.

lead to enhanced signaling via Jak/Stat or PKC. These pathways regulate gene transcription and thereby modulate cell proliferation, apoptosis, angiogenesis, and malignant transformation. Two strategies for blocking the action of these receptors include antibodies directed against the ectodomain and drugs that inhibit protein-tyrosine kinase activity. A reversible competitive inhibitor of EGFR (gefitinib) and an EGFR ectodomain directed antibody (cetuximab) have been approved for the treatment of non–small-cell lung cancer (NSCLC) and colorectal cancer, respectively.[93] An ErbB-2/HER2 ectodomain-directed antibody (trastuzumab) has also been approved for the treatment of breast cancer.[94]

There is increasing evidence that EGFR plays a significant role in tumor formation and early disease, leading to the hypothesis that EGFR could be an attractive pharmacologic target for chemoprevention. This hypothesis has been strengthened by recent preclinical studies with EGFR tyrosine kinase inhibitors. Several studies have suggested that targeting EGFR may protect against the development of invasive breast cancer. Lenferink et al[95] showed that inhibition of the EGFR tyrosine kinase with AG-1478 markedly delayed breast tumor formation in experimental animals. This delay was associated with inhibition of EGFR and *Neu* signaling, reduction of MAPK activity and cyclin D1 levels, and an increase in amounts of the cyclin-dependent kinase inhibitor p27. Importantly, new approaches are needed to prevent estrogen receptor–negative breast cancer. Expression of EGFR is inversely correlated with the expression of the estrogen receptor in breast carcinomas.[96] Recently, gefitinib was found to suppress mammary tumorigenesis in MMTV-ErbB-2 transgenic mice, a model of estrogen receptor–negative breast cancer.[97] Notably, treatment with gefitinib led to a 20.3% reduction in proliferation of normal breast cells and a 42% reduction in the proliferation of tumor cells. In another study, Chan et al[98] reported that an EGFR tyrosine kinase inhibitor potently inhibited the proliferation of EGFR-positive ductal carcinoma-in-situ transplanted as xenografts into nude mice. Thus, targeting EGFR tyrosine kinase represents a potential chemopreventive approach in patients with ductal carcinoma-in-situ who are at high risk for developing invasive breast cancer.

As mentioned above, colorectal adenomas are premalignant lesions, and their presence increases the risk for development of colorectal cancer. Strategies for successful prevention of colorectal cancer must include the removal or chemoprevention of colorectal adenomas. Complementary studies using pharmacologic and genetic approaches suggest that targeting EGFR is a rational approach to reduce the risk of intestinal tumorigenesis. Treatment with EKB-569 or EKI-785, irreversible inhibitors of EGFR tyrosine kinase, reduced

intestinal polyp number in the Apc^{Min} mouse model of FAP.[3] Recently, *Egfr*^{wa2} mice have been developed in which there is a marked reduction in EGFR kinase activity due to a point mutation in the kinase domain of the receptor. Transfer of the Apc^{Min} allele onto a homozygous *Egfr*^{wa2} background resulted in a 90% reduction in intestinal polyp number relative to APC^{Min} mice that were wild-type for the EGFR.[99] Taken together, these findings indicate an important role for EGFR in early intestinal carcinogenesis.

Preclinical studies also support the notion of targeting EGFR to prevent or delay the onset of upper aerodigestive malignancies. Aberrant EGFR expression is common in bronchial metaplasia suggesting a potential link between EGFR and the development of NSCLC.[100,101] In a model of human bronchial carcinogenesis, Lonardo et al[102] used retinoic acid as a tool to demonstrate the potential of EGFR as a chemoprevention target in lung cancer. These findings helped to provide a rationale for a proposed phase III lung cancer prevention trial of gefitinib versus placebo in former/current smokers with a previous history of cancer.[103] Other studies have suggested a role for targeting EGFR in the prevention of head and neck squamous cell carcinoma. For example, EGFR expression is deregulated in oral mucosa during head and neck tumorigenesis.[104,105] Moreover, in model systems, we have shown that clinically achievable concentrations of an EGFR tyrosine kinase inhibitor block the growth of aneuploid oral leukoplakia cells. Taken together, the above findings strengthen the rationale for carrying out clinical trials to determine whether targeting EGFR will reduce the risk of aerodigestive malignancies in high-risk individuals (see below).

As mentioned above, targeting EGFR can be of benefit in the treatment of some aerodigestive malignancies. However, both skin rash and gastrointestinal toxicity are quite common in the treatment setting.[106] In the context of chemoprevention, tolerability will be a more important factor than it is in the advanced disease setting. Hence, the success or failure of targeting EGFR as an approach to chemoprevention for any premalignant condition will depend heavily on the balance between efficacy and tolerability.

CROSSTALK BETWEEN EGFR AND COX-2

As detailed above, both COX-2 and EGFR are promising targets for chemoprevention. In this context, it is important to review the evidence of crosstalk between EGFR and COX-2. Activation of EGFR signaling leads to increased MAPK activity resulting, in turn, in AP-1–mediated induction of *COX-2* transcription (Fig 2).[2] Increased *COX-2* transcription results in enhanced production of PGs, including PGE_2. Notably, there also is growing evidence that COX-2–derived PGE_2 can activate EGFR signaling. Several recent studies have found that PGE_2 can activate EGFR signaling and thereby stimulate cell proliferation.[107-109] The mechanism(s) by which this occurs appear to be complex and context specific. In one study, the ability of PGE_2 to transactivate EGFR was rapid and depended on matrix metalloproteinase activity.[107] PGE_2 activated metalloproteinase activity resulting in shedding of active EGFR ligand from the plasma membrane. This led, in turn, to increased EGFR signaling and enhanced DNA synthesis. In another study, treatment with PGE_2 activated the cAMP/protein kinase A pathway leading to increased expression of amphiregulin, a ligand of EGFR (Fig 3).[108] PGE_2 also has been observed to transactivate EGFR via an intracellular Src-mediated event independent of the release of an extracellular ligand of EGFR (Fig 3).[109] Regardless of the precise mechanism, exposure to COX-2-derived PGE_2 may initiate a positive feedback loop whereby activation of EGFR results in enhanced expression of COX-2 and increased synthesis of PGs. This leads, in turn, to a further enhancement of EGFR activity.

Recently, we investigated whether this mechanism might be relevant in cigarette smokers.[110] First, we found an approximately four-fold increase in amounts of COX-2 in the oral mucosa of active smokers compared with never smokers. Subsequently, we demonstrated that treatment of a human oral epithelial cell line with a tobacco smoke extract stimulated *COX-2* transcription resulting in increased PGE_2 synthesis (Fig 5). Several findings support a critical role for EGFR signaling in tobacco smoke–mediated induction of COX-2. Treatment with tobacco smoke extract stimulated the phosphorylation of EGFR. Moreover, antibody-mediated blockade of the ligand-binding site of EGFR or treatment with an inhibitor of EGFR tyrosine kinase abrogated tobacco smoke–mediated induction of COX-2. The ability of tobacco smoke to activate EGFR was mediated, at least in part, by matrix metalloproteinase–dependent ectodomain shedding of EGFR ligands (Fig 5). Therefore, exposure to tobacco smoke may initiate a positive feedback loop whereby activation of EGFR results in enhanced expression of COX-2 and increased synthesis of PGs. This leads, in turn, to a further enhancement of EGFR activity. These effects could enhance the mutagenicity of tobacco smoke. For example, COX-2 can convert a broad array of carcinogens, including polycyclic aromatic hydrocarbons in tobacco smoke, to reactive metabolites, which form mutagenic DNA adducts.[111,112] It is possible, therefore, that tobacco smoke–mediated induction of COX-2 will amplify the effect of a given dose of tobacco smoke on tumor initiation. Moreover, conversion of DNA adducts to mutations can only occur in proliferating cells[113,114] and activation of EGFR signaling or stimulation of PG biosynthesis enhances cell proliferation[43,44] which should then increase the mutagenicity of tobacco smoke. These findings strengthen the rationale for evaluating whether a selective inhibitor of COX-2 or EGFR tyrosine kinase used alone or in combination can prevent or

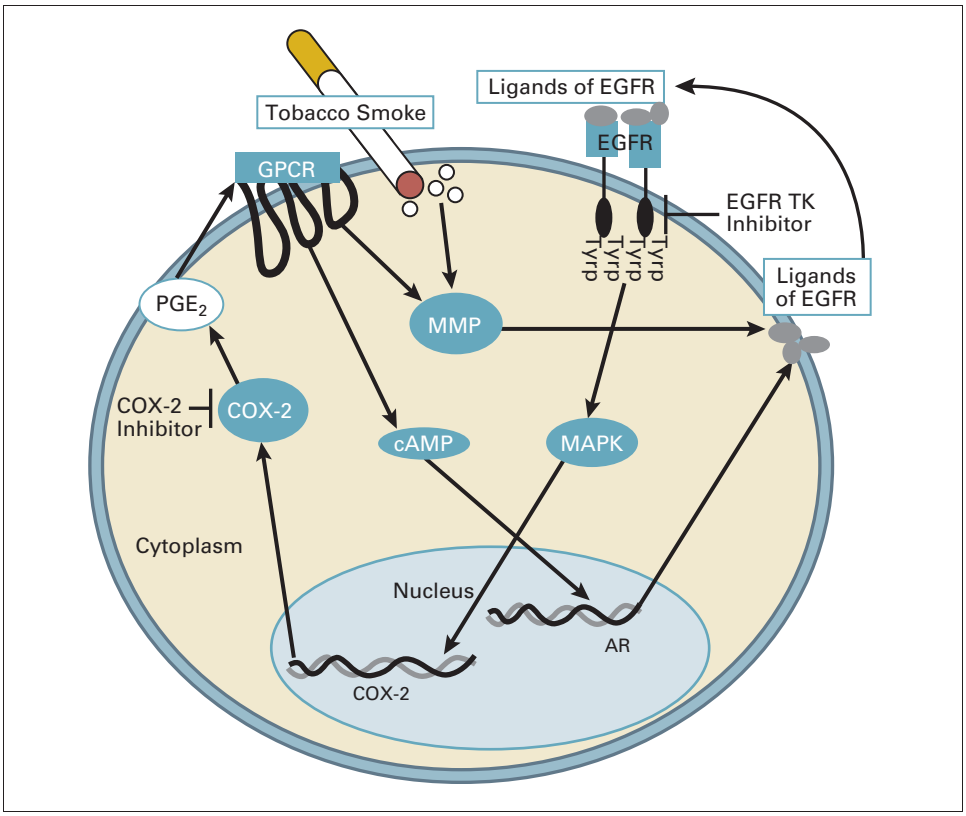

Fig 5. Tobacco smoke-mediated induction of cyclooxygenase-2 (COX-2) is dependent on activation of epidermal growth factor receptor (EGFR). Tobacco smoke stimulates the rapid release of EGFR ligands from the plasma membrane resulting in activation of EGFR. This leads, in turn, to enhanced mitogen-activated protein kinase (MAPK) activity, increased *COX-2* transcription and stimulation of prostaglandin E_2 (PGE_2) biosynthesis. PGE_2, in turn, can activate a G protein coupled receptor (GPCR) and thereby stimulate amphiregulin (AR) transcription or matrix metalloproteinase (MMP) -dependent release of EGFR ligands. Collectively, these effects amplify both the stimulation of EGFR signaling and induction of *COX-2* transcription. Treatment with a selective COX-2 inhibitor, an EGFR tyrosine kinase inhibitor or the combination of these two agents may reduce the procarcinogenic effects of tobacco smoke.

delay the onset of tobacco smoke–related malignancies of the aerodigestive tract (see below).

COMBINATION CHEMOPREVENTION

Combination therapy, a common strategy in cancer treatment, might also be applicable to chemoprevention. Little is known, however, about whether this approach will be either effective or sufficiently free of side effects to be useful in chemoprevention. As detailed above, both EGFR signaling and COX-2 expression are frequently deregulated in neoplasia. Activation of either EGFR signaling or increased production of COX-2–derived PGs can impact several mechanisms that have been linked to carcinogenesis, including cell proliferation, apoptosis, and angiogenesis. Moreover, crosstalk between EGFR and COX-2 can potentially amplify the carcinogenic process. Preclinical studies suggest that combining an inhibitor of EGFR tyrosine kinase with an inhibitor of COX-2 will be more effective than using either agent alone. For example, combining a selective COX-2 inhibitor with the EGFR tyrosine kinase inhibitor, gefitinib, was more effective than either agent alone in suppressing experimental tumor growth.[115] Torrance et al[3] addressed this point by evaluating the number of adenomas that develop in Apc^{Min} mice after treatment with the NSAID sulindac and EKB-569, an inhibitor of EGFR tyrosine kinase. Apc^{Min} mice, which normally develop numerous intestinal polyps as a result of a mutation in the *APC* tumor suppressor gene, were almost completely protected from adenomas after treatment with EKB-569 and sulindac. The significant reduction in adenomas seen with the combination of sulindac and EKB-569 raises the question of whether such an approach will be clinically useful. Toxicity, including diarrhea or skin rash, could develop during prolonged use of an EGFR tyrosine kinase inhibitor.[106] Perhaps low doses of combinations of agents will be more effective than either agent alone, and have reduced toxicity. As previously stated, individuals at extremely high risk of developing malignancies will be much more willing to risk experiencing side effects related to treatment than individuals at average risk for cancer. Given this consideration, a logical next step will be to determine whether the combination of an inhibitor of EGFR tyrosine kinase and a selective COX-2 inhibitor is more effective than either agent alone in preventing or delaying the development of cancer in high-risk individuals.

TARGETING COX-2 AND EGFR IN A CLINICAL CHEMOPREVENTION TRIAL

A phase III randomized placebo-controlled trial using a 2 × 2 factorial design in patients with aneuploid dysplastic oral leukoplakia is in advanced planning stages before activation

(Fig 6). Three hundred randomly assigned, eligible patients will be treated for 1 year in four arms of 75 patients each: the EGFR inhibitor EKB-569 (25 mg qd) alone, COX-2-inhibitor celecoxib (400 mg bid) alone, EKB-569 plus celecoxib, or placebo. The primary end point is the development of oral cancer. Based on the extreme cancer risk of aneuploid oral leukoplakia patients (discussed in the next paragraph), this trial will have over an 80% power to detect a 40% cancer reduction. The secondary end points include multiple molecular end points based on EGFR and COX-2 signaling, safety, and pharmacogenetic information. Patient accrual will take place in Norway, Sweden, Denmark, and Finland, where an estimated 4,500 patients with oral leukoplakia will be screened for cancer risk during the 2- to 3-year accrual period. Patients with aneuploid dysplastic oral leukoplakia are entered into the Nordic national tumor registries, which are unusual because they register and track premalignant lesions, and will be potentially eligible for the clinical trial.

These patients have over a 75% risk of biologically aggressive oral cancer in a little less than 7 years (mean, 80 months).[116-118] This very high risk (discussed in detail in Lippman et al in this issue of the *Journal of Clinical Oncology*[119]) permits the use of cancer development rather than leukoplakia response as the primary end point and permits the important secondary assessment of the validity of oral leukoplakia as a surrogate end point for cancer development (ie, the correlation between oral leukoplakia response to treatment and cancer development), unique features of any clinical trial in the setting of oral leukoplakia. Observational data, however, indicate that complete resection of oral leukoplakia lesions does not prevent the development of clinically aggressive carcinoma.[116] The extreme cancer risk of aneuploid oral leukoplakia patients makes this trial tantamount to a cancer therapy trial, illustrating an important new direction in clinical cancer drug development, the convergent development of drugs for prevention and therapy.[120] This convergence is further illustrated by the ongoing development of the trial agents celecoxib and EKB-569 for both cancer prevention and therapy.

Celecoxib and EKB-569 were chosen for this trial primarily because their targets (COX-2 and EGFR, respectively) are expressed in aneuploid oral leukoplakia and are considered important factors in oral carcinogenesis. The doses of celecoxib and EKB-569 for this trial are based on strong preclinical and clinical evidence of activity and safety. Regarding celecoxib, we chose the relatively aggressive dose of 400 mg bid rather than a lower dose because of the therapeutic demands associated with the extremely high-risk patients in this trial. The activity of celecoxib (400 mg bid) includes significantly reducing colorectal polyp burden (leading to its approval by the United States Food and Drug Administration as adjunctive therapy for the management of polyps) in FAP patients[79]; inhibiting its target in pharmacodynamic studies within both NSCLC[121] and oral leukoplakia[122]; and inhibiting proliferation and inducing apoptosis in responsive FAP patients' adenomas.[123] This dose also has been shown to be well tolerated in clinical trials in other settings. Regarding EKB-569: the dose 25 mg/d produces plasma levels in humans that are similar to the levels shown to be active in head and neck cancer cells in vitro and head and neck cancer xenografts; is well below the maximum-tolerated dose (75 mg/d); is one half the recommended phase II therapy dose (50 mg/d); and has been shown to suppress pEGFR and pERK1/ERK2 levels in skin biopsies from a phase I trial (personal communication, Lee Greenberger, September 2003).[124] Regarding combined EKB-569 and celecoxib, the combination is expected to have enhanced activity based on the two agents' interactive signaling pathways. The combination is not expected to have enhanced toxicity (versus either single agent alone), however, because each agent has a different molecular mechanism and target; the single-agent toxicities are nonoverlapping, and there is a limited

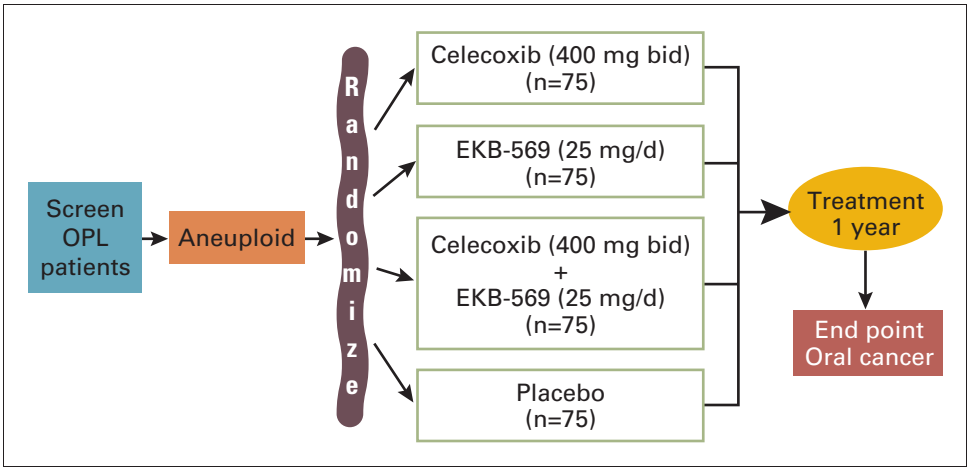

Fig 6. Clinical trial schema. This schema represents a planned phase III randomized placebo-controlled trial in patients with aneuploid dysplastic oral leukoplakia. The cyclooxygenase-2 (COX-2) inhibitor celecoxib and epidermal growth factor receptor (EGFR) inhibitor EKB-569 will be tested in a 2 × 2 factorial design.

potential for pharmacokinetic interactions since the primary metabolism of each study agent is distinct from the other. Notwithstanding the strong safety rationale supporting this trial, toxicity will be monitored very rigorously to ensure patient safety to the greatest extent possible.

CONCLUSION

Significant progress has been made in defining the link between COX-2, EGFR, and carcinogenesis, but many important questions remain unanswered. First and foremost, it will be important to establish whether selective COX-2 inhibitors are effective in preventing or delaying the onset of cancer. Because numerous clinical trials are underway, valuable information should be forthcoming in the not too distant future. Selective COX-2 inhibitors have been extensively used to treat arthritis and pain for several years. Hence, epidemiologic studies are anticipated that should provide additional insights about the relationship between use of selective COX-2 inhibitors and the relative risk of developing a spectrum of malignancies.[125]

Selective COX-2 inhibitors have an excellent safety profile when given as monotherapy to arthritis patients. However, concerns have been raised about the cardiovascular safety of selective COX-2 inhibitors.[126] In the VIGOR trial, the incidence of myocardial infarction was significantly higher in groups treated with rofecoxib versus naproxen.[126] Whether this difference reflected a chance event, a prothrombotic effect of rofecoxib, or a cardioprotective effect of naproxen is uncertain. Importantly, this does not appear to be a class effect because similar effects have not been observed in studies of celecoxib.[127] Ongoing placebo-controlled trials, including the colorectal adenoma prevention trials, will provide additional useful safety data.

Genetic studies using either transgenic or knockout technology have firmly established the link between COX-2 and tumorigenesis.[63-66] Whether pharmacologic inhibitors of COX-2 suppress tumorigenesis exclusively by inhibiting COX-2 is less certain.[128] For example, high concentrations of NSAIDs or selective COX-2 inhibitors suppress the growth of cells in culture that do not express COX-2.[129] It is possible, therefore, that the anticancer activity of NSAIDs and selective COX-2 inhibitors may also reflect COX-independent effects. Other possible pharmacologic targets have been identified including cGMP phosphodiesterases, PPARs, NF-κB, Akt, and 15-lipoxygenase-1.[128] A question of major importance will be to determine which, if any, of these COX-independent effects occur in humans given clinically relevant doses of a selective COX-2 inhibitor.

To be useful in humans, a chemopreventive agent needs to have an acceptable therapeutic index. Side effects of inhibitors of EGFR tyrosine kinase have limited enthusiasm for evaluating these agents in chemoprevention trials. It will be extremely important, therefore, to assess both the efficacy and safety of EKB-569 in the planned aneuploid leukoplakia clinical trial. Another potential approach to reducing toxicity is to administer inhibitors of EGFR tyrosine kinase topically. For example, our discovery that tobacco smoke exposure enhances EGFR tyrosine kinase activity raises the possibility that topical administration of an inhibitor might reduce the risk of aerodigestive malignancy without causing significant systemic side effects. In evaluating chemopreventive agents, the development of surrogate end point biomarkers that provide objective evidence of clinical benefit remains extremely challenging. In the meantime, trials with a cancer end point such as the one described above in patients with aneuploid leukoplakia should provide critical insights.

Editor's Note

Since the acceptance for publication of the revised manuscript, rofecoxib was voluntarily withdrawn from the world market because of an increased number of thromboembolic events (acute myocardial infarction and stroke) in persons enrolled in a colorectal polyp prevention trial. These side effects were detected in the group treated with rofecoxib (25 mg per day) after 18 months of treatment. As of today (December 8, 2004), there has been no imbalance of thromboembolic events between the celecoxib and placebo arms observed in ongoing analyses of large-scale, randomized, placebo-controlled trials of celecoxib, including polyp prevention trials designed similarly to the rofecoxib trial.

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Authors' Disclosures of Potential Conflicts of Interest

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