Levels of Cyclooxygenase-2 Are Increased in the Oral Mucosa of Smokers: Evidence for the Role of Epidermal Growth Factor Receptor and Its Ligands

Dimitrios Moraitis,1 Baoheng Du,2 Mariana S. De Lorenzo,2 Jay O. Boyle,1 Babette B. Weksler,2 Erik G. Cohen,1 John F. Carew,1 Nasser K. Altorki,1 Levy Kopelovich,5 Kotha Subbaramaiah,2 and Andrew J. Dannenberg1

1Head and Neck Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center; Departments of Medicine, Otorhinolaryngology, and Cardiothoracic Surgery, Weill Medical College of Cornell University, New York, New York; and 3Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Abstract

Cyclooxygenase-2 (COX-2) is a promising pharmacologic target for preventing aerodigestive malignancies. In this study, we investigated the effects of tobacco smoke on the expression of COX-2 in oral mucosa. An ~4-fold increase in amount of COX-2 mRNA was observed in the oral mucosa of active smokers versus never smokers. Thus, a series of in vitro studies were carried out to elucidate the mechanism by which tobacco smoke induced COX-2. Treatment of a nontumorigenic oral epithelial cell line (MSK-Leuk1) with a saline extract of tobacco smoke (TS) stimulated COX-2 transcription, resulting in increased amounts of COX-2 mRNA, COX-2 protein, and prostaglandin E2 (PGE2) synthesis. Exposure of cells to TS also caused an increase in epidermal growth factor receptor (EGFR) tyrosine kinase activity. Both an inhibitor of EGFR tyrosine kinase activity and a neutralizing anti-EGFR antibody blocked TS-mediated induction of COX-2. To define the mechanism by which TS activated EGFR, the release of amphiregulin and transforming growth factor α (TGFα), two ligands of the EGFR, was measured. Exposure to TS caused a rapid increase in the release of both ligands. TS also markedly induced the expression of mRNAs for amphiregulin and transforming growth factor α. Importantly, increased expression of both ligands was also detected in the oral mucosa of active smokers. Taken together, these results suggest that activation of EGFR signaling contributes to the elevated levels of COX-2 found in the oral mucosa of smokers. Moreover, these findings strengthen the rationale for determining whether inhibitors of COX-2 or EGFR tyrosine kinase activity can reduce the risk of tobacco smoke–related malignancies of the aerodigestive tract. (Cancer Res 2005; 65(2): 664-70)
Materials and Methods

**Materials.** DMEM/Ham’s F-12 medium and fetal bovine serum were from Life Technologies, Inc. (Grand Island, NY). Keratinocyte basal and growth media were from Clonetics Corp. (San Diego, CA). Sodium arachidonate and kits for lactate dehydrogenase and Lowry protein measurements were from Sigma Chemical Co. (St. Louis, MO). Enzyme immunoassay reagents for PGE$_2$ assays and COX-2 cDNA were from Cayman Chemical Co. (Ann Arbor, MI). Complete mini protease inhibitor mixture was from Roche Diagnostics (Mannheim, Germany). Enzyme immunoassay kits for amphiregulin and TGF-α were purchased from R&D Systems, Inc. (Minneapolis, MN) and Oncogene Research Products (San Diego, CA), respectively. Amphiregulin and TGF-α were obtained from R&D Systems, Inc. [α-32P]dCTP was from Perkin-Elmer Life Sciences (Boston, MA). Random-priming kits were from Boehringer-Mannheim Biochemicals (Indianapolis, IN). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). RNA was prepared using kits from Qiagen (Chatsworth, CA). The 18S rRNA cDNA was from Ambion, Inc. (Austin, TX). Anti-human COX-2 polyclonal antibodies and anti-phosphotyrosine antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibodies to EGFR were purchased from Upstate (Lake Placid, NY). Western blotting detection reagents were from Amersham Biosciences (Piscataway, NJ). AG1478 was purchased from Calbiochem (La Jolla, CA).

**Human Tissue.** Buccal mucosa specimens were obtained from 9 never smokers and 10 active smokers (≥10 pack-years). Subjects were excluded if they had good evidence of oral inflammation, a history of heavy alcohol consumption, or recent use of nonsteroidal anti-inflammatory drugs or other anti-inflammatory medications. After topical anesthesia, 3-mm punch biopsies were obtained from grossly normal appearing buccal mucosa. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at −80°C until analysis. This study was approved by the Committee on Human Rights in Research at Weill Medical College of Cornell University.

**Tissue Culture.** The MSK-Leuk1 cell line was established from a dysplastic leukoplakia lesion adjacent to a squamous cell carcinoma of the tongue (35). Cells were routinely maintained in keratinocyte growth medium supplemented with bovine pituitary extract. Human 1483 squamous carcinoma cells have been described previously (36). The non-small cell lung cancer cell line H2122 was a generous gift of Dr. Raphael A. Nemenoff (University of Colorado, Denver, CO; ref. 37). Cells were grown in basal medium for 24 hours before treatment. Treatment with vehicle (PBS) or a saline extract of tobacco smoke (TS, see below) was carried out under serum-free conditions. Cellular cytotoxicity was assessed by measurements of cell number, release of lactate dehydrogenase and trypan blue exclusion. There was no evidence of cytotoxicity in any of the experiments.

**Preparation of Tobacco Smoke.** Cigarettes (Marlboro, King Size) were smoked in a Borgwaldt piston-controlled apparatus (Model RG-1, Hamburg, Germany) using the Federal Trade Commission standard protocol. The protocol parameters attempt to mimic a standardized human smoking pattern (duration, 2 seconds/puff; frequency, 1 puff/min; volume, 35 mL/puff). Cigarettes were smoked one at a time in the apparatus and the smoke drawn under sterile conditions into premeasured amounts of sterile PBS, pH 7.4. This smoke in PBS represents whole trapped mainstream smoke, abbreviated as TS. Quantitation of smoke content is expressed in puffs/mL of PBS with one cigarette yielding about 8 puffs drawn into a 5 mL volume. The final concentration of TS in the cell culture medium is expressed as puffs per mL medium.

**PGE$_2$ Production.** Cells (5 × 10$^4$ per well) were plated in six-well dishes and grown to 60% confluence in growth medium. At the end of the treatment period, the medium was replaced with fresh medium containing 10 μmol/L sodium arachidonate. Thirty minutes later, the medium was collected for analysis of PGE$_2$. Production of PGE$_2$ was determined by enzyme immunoassay according to the manufacturer’s instructions. Amounts of PGE$_2$ are expressed as picograms per microgram cellular protein.

**Immunoprecipitation and Western Blotting.** For EGFR, immunoprecipitates were prepared by treating cells with lysis buffer [20 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 50 mmol/L NaF, 30 mmol/L sodium pyrophosphate, 5 mmol/L EGTA, 10% glycerol, 1% Triton X-100, 1 mmol/L phenylmethanesulfonylfluoride, 1 mmol/L Na$_3$VO$_4$, 5 μg/mL aprotinin, Complete mini protease inhibitor mixture, and 5 μmol/L 3,4 dichlorocoumarin]. Lysates were sonicated for 20 seconds on ice and centrifuged at 10,000 × g for 10 minutes to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry et al. (38). The supernatant was preabsorbed with 20 μL of normal goat immunoglobulin G at 4°C; 80 μL of protein G PLUS-agarose was then added. The mixture was then centrifuged at 3,000 × g for 5 minutes at 4°C. The pellet was discarded. Four microliters of monoclonal anti-human EGFR antisera was added to the supernatant; the mixture was then incubated at 4°C on a rocker platform overnight. Eighty microliters of protein G PLUS-agarose was then added, and the mixture was then incubated at 4°C on a rocker platform for 2 hours; the mixture was then centrifuged at 3,000 × g for 5 minutes at 4°C. The supernatant was discarded. After washing the pellet four times with lysis buffer, the pellet was resuspended. Cell lysates for COX-2 analysis were prepared as previously described (39).

**SDS-PAGE was then done under reducing conditions on 7.5% or 10% polyacrylamide gels. The resolved proteins were transferred onto nitrocellulose sheets. The nitrocellulose membrane was then incubated with primary antisera to phosphotyrosine, EGFR, COX-2, or β-actin. Secondary antibody to immunoglobulin G conjugated to horseradish peroxidase was used. The blots were then reacted with enhanced chemiluminescence Western blot detection system according to the manufacturer’s instructions.**

**Northern Blotting.** Total cellular RNA was isolated from cell monolayers using the RNA isolation kit from Qiagen. Ten micromgrams of RNA per lane were electrophoresed in a formaldehyde-containing 1% agarose gel and transferred to nylon-supported membranes. The membrane was hybridized with radiolabeled cDNA probes for human COX-2 and 18S rRNA, respectively, as described previously (3, 5, 39).

**Transient Transfection Assays.** Cells were seeded at a density of 5 × 10$^4$ cells/well in six-well dishes and grown to 50% to 60% confluence.
Tobacco smoke induces COX-2 protein and PGE₂ synthesis in cell lines derived from the human aerodigestive tract. MSK-Leuk1 (A and E), 1483 cells (B and D), and H2122 (C) cells were treated with TS for 6 to 24 hours. A, lysate protein was from MSK-Leuk1 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), TS (0.030 puffs/mL, lane 4) or TS (0.06 puffs/mL) for 12 hours. B, lysate protein was from 1483 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), or TS (0.030 puffs/mL, lane 4) for 24 hours. C, lysate protein was from H2122 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), or TS (0.030 puffs/mL, lane 4) for 24 hours. D, lysate protein was from 1483 cells treated with vehicle (lanes 1, 5, and 9), TS (0.0075 puffs/mL, lanes 2, 6, and 10), TS (0.015 puffs/mL, lanes 3, 7, and 11), or TS (0.030 puffs/mL, lanes 4, 8, and 12) for 6 hours (lanes 1-4), 12 hours (lanes 5-8), and 24 hours (lanes 9-12), respectively. In A to D, cellular lysate protein (100 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed sequentially for COX-2 and β-actin. E, MSK-Leuk1 cells were treated with vehicle or the indicated concentration of TS for 12 hours. The medium was then replaced with fresh basal medium and 10 μmol/L sodium arachidonate. Thirty minutes later, the medium was collected to determine COX-2 activity. Production of PGE₂ was determined by enzyme immunoassay. Columns, means (n = 6); bars, SD. *, P < 0.05; ** P < 0.01.

Figure 2. Tobacco smoke induces COX-2 protein and PGE₂ synthesis in cell lines derived from the human aerodigestive tract. MSK-Leuk1 (A and E), 1483 cells (B and D), and H2122 (C) cells were treated with TS for 6 to 24 hours. A, lysate protein was from MSK-Leuk1 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), TS (0.030 puffs/mL, lane 4) or TS (0.06 puffs/mL) for 12 hours. B, lysate protein was from 1483 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), or TS (0.030 puffs/mL, lane 4) for 24 hours. C, lysate protein was from H2122 cells treated with vehicle (lane 1), TS (0.0075 puffs/mL, lane 2), TS (0.015 puffs/mL, lane 3), or TS (0.030 puffs/mL, lane 4) for 24 hours. D, lysate protein was from 1483 cells treated with vehicle (lanes 1, 5, and 9), TS (0.0075 puffs/mL, lanes 2, 6, and 10), TS (0.015 puffs/mL, lanes 3, 7, and 11), or TS (0.030 puffs/mL, lanes 4, 8, and 12) for 6 hours (lanes 1-4), 12 hours (lanes 5-8), and 24 hours (lanes 9-12), respectively. In A to D, cellular lysate protein (100 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed and subsequently transferred onto nitrocellulose. Immunoblots were probed sequentially for COX-2 and β-actin. E, MSK-Leuk1 cells were treated with vehicle or the indicated concentration of TS for 12 hours. The medium was then replaced with fresh basal medium and 10 μmol/L sodium arachidonate. Thirty minutes later, the medium was collected to determine COX-2 activity. Production of PGE₂ was determined by enzyme immunoassay. Columns, means (n = 6); bars, SD. *, P < 0.05; ** P < 0.01.

Figure 3. Tobacco smoke induces COX-2 mRNA and stimulates COX-2 promoter activity. A, MSK-Leuk1 cells were treated with vehicle or TS (0.030 puffs/mL) for 2 or 4 hours. B, 1483 cells were treated with vehicle (lane 1) or the indicated concentration of TS for 4 hours. Total cellular RNA was isolated; 10 μg of RNA was added to each lane. The Northern blot was probed sequentially for COX-2 mRNA and 18S rRNA. C, MSK-Leuk1 cells were transfected with the −1432/+59 COX-2 promoter. After transfection, cells were treated with vehicle or the indicated concentration of TS. Reporter activities were measured in cellular extracts 12 hours after treatment. Luciferase activity represents data that have been normalized to β-galactosidase activity. Columns, means (n = 6); bars, SD. *, P < 0.05; **, P < 0.01 compared with vehicle-treated control.
25°C, 15 minutes at 42°C, 5 minutes at 99°C, and 5 minutes at 5°C. The resulting cDNA was then used for amplification. The volume of the PCR reaction was 25 μL and contained 2 μL cDNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2 mmol/L MgCl2, 0.4 mmol/L deoxynucleotide triphosphates, 400 nmol/L forward primer, 400 nmol/L reverse primer, and 2.5 units Taq polymerase (Applied Biosystems, Foster City, CA). Samples were denatured at 95°C for 2 minutes and then amplified for 35 cycles in a thermocycler under the following conditions: 95°C for 10 minutes. Primer sequences were as follows: amphiregulin, forward 5'-AAGCGTGAAACCATTTTCTGG-3', reverse 5'-TTTCTATGGACTTTTCCCAC-3'; TGF-α, forward 5'-GTGTGCAAGCAGTGGTGCTT-3', reverse 5'-CCCAAGCCTTAGCTGTCTTG-3'; HB-EGF, forward 5'-GTTGGTGCTGAAGCTCTTTCC-3', reverse 5'-CCTAAACATGAAGCCCAAC-3'; and β-actin, forward 5'-GGTCACCCACACTGTGCCCAT-3', reverse 5'-GGATGCCACAGGACTCCATGC-3'. To determine levels of mRNAs for amphiregulin and TGF-α, reverse transcription–PCR (RT-PCR) was carried out. Treatment with TS caused dose-dependent induction of COX-2 protein in a nontumorigenic cell line derived from a premalignant oral lesion (MSK-Leuk1). Notably, TS-mediated induction of COX-2 protein was associated with increased synthesis of PGE2.

To further elucidate the mechanism responsible for changes in amounts of COX-2 protein, we measured steady-state levels of COX-2 mRNA by Northern blotting (Fig. 3). The results confirmed by DNA sequencing. A computer densitometer (Chem Doc; Bio-Rad, Hercules, CA) was used to quantify the density of the different bands. Comparisons between groups were made by the Student's t test. A difference between groups of P < 0.05 was considered significant.

**Results**

Quantitative reverse transcription–PCR (RT-PCR) was carried out to determine amounts of COX-2 mRNA in the buccal mucosa of smokers (n = 10) versus never smokers (n = 9). A nearly 4-fold increase in mean levels of COX-2 message was detected in smokers (Fig. 1). To determine whether this might reflect a direct effect of tobacco smoke, in vitro studies were carried out. Treatment with TS caused dose-dependent induction of COX-2 mRNA in two other cell lines (1483 and H2122) derived from the human aerodigestive tract. TS induced COX-2 in each of these cell lines (Fig. 2B and C). Induction of COX-2 was observed after 6 hours of exposure to TS and persisted for at least 24 hours (Fig. 2D).

**Figure 4.** Tobacco smoke induces COX-2 by an EGFR-dependent mechanism. A, MSK-Leuk1 cells were treated with vehicle or TS (0.030 puffs/mL) for up to 8 hours. Cell lysates were immunoprecipitated (IP) with anti-EGFR antibody, immunoblotted (WB) with anti-phosphotyrosine antibody (PY, top) or anti-EGFR antibody (bottom), and visualized with chemiluminescence. B, MSK-Leuk1 cells were pretreated with vehicle or the indicated concentration of AG1478 for 2 hours. Subsequently, cells were treated with vehicle, TS (0.030 puffs/mL), or TS plus the indicated concentration of AG1478 for 12 hours. C, MSK-Leuk1 cells were pretreated for 2 hours with vehicle, EGFR-neutralizing antibody (10 μg/mL), or mouse immunoglobulin G (10 μg/mL). Subsequently, cells were treated with vehicle or TS (0.030 puffs/mL) for 12 hours. In B and C, cellular lysate protein (100 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed sequentially for COX-2 and β-actin.

**Figure 5.** Tobacco smoke induces the expression of EGFR ligands in MSK-Leuk1 cells. A, cells were treated with vehicle or TS (0.030 puffs/mL) for up to 4 hours. Amounts of amphiregulin (AR) and TGF-α protein in the medium were determined at the indicated time points by enzyme immunoassay. Columns, means (n = 6); bars, SD. **, P < 0.01 compared with vehicle. B, cells were treated with vehicle or TS (0.030 puffs/mL) for up to 16 hours. RT-PCR analysis was done at different points for AR, TGF-α, HB-EGF, and β-actin mRNAs. STD, standard.
formation of COX-2 mRNA. Differences in levels of COX-2 mRNA could reflect altered rates of transcription. Transient transfections were carried out to determine the effects of TS on COX-2 promoter activity (Fig. 3C). Treatment with TS led to approximately a tripling of COX-2 promoter activity.

Activation of several different signaling cascades including the EGFR→Ras pathway leads to increased COX-2 expression (42). Given this background, we investigated whether TS-mediated induction of COX-2 was EGFR dependent. Accordingly, the effects of TS on levels of phospho-EGFR were measured. TS stimulated EGFR tyrosine kinase activity (Fig. 4A). Subsequently, experiments were carried out to determine whether the activation of EGFR was causally linked to TS-mediated induction of COX-2. Importantly, an inhibitor of EGFR tyrosine kinase activity (AG1478) blocked TS-mediated induction of COX-2 (Fig. 4B). This implies that the induction of COX-2 by TS is dependent on EGFR signaling. Next it was important to begin to define the mechanism by which TS activated EGFR. Activation of EGFR can be growth factor dependent in the absence of exogenous growth factor. Hence, we investigated whether ligand binding was involved in TS-mediated stimulation of EGFR tyrosine kinase activity. We found that antibody blockade of the ligand-binding site abrogated TS-mediated induction of COX-2 (Fig. 4C). This finding can potentially be explained if TS treatment stimulated the release of EGFR ligands. In fact, treatment with TS led to increased amounts of both amphiregulin and TGF-α protein in the medium (Fig. 5A). This effect occurred rapidly, within 1 hour of TS exposure. RT-PCR was carried out to determine whether the observed increase in amounts of amphiregulin and TGF-α protein in the medium could reflect at least, in part, enhanced gene expression. Exposure to TS led to a marked increase in amounts of amphiregulin and TGF-α mRNAs (Fig. 5B). However, TS-mediated induction of amphiregulin mRNA (Fig. 5B) was first observed only after 2 hours of treatment, whereas

Figure 6. Increased levels of amphiregulin and TGF-α are detected in the buccal mucosa of smokers. Biopsies of buccal mucosa were obtained from 9 never smokers and 10 active smokers (>10 pack-years). A, RT-PCR was used to determine the amounts of mRNAs for AR and TGF-α in these samples. B, results of the data shown in A expressed in arbitrary units (a.u.). Columns, means; bars, SD. *, P < 0.05; **, P < 0.01 compared with never smokers.

Figure 7. Treatment with either amphiregulin or TGF-α induced COX-2. A, lysate protein was from MSK-Leuk1 cells treated with vehicle or the indicated concentration of AR (1-25 ng/mL) for 6 hours. B, lysate protein was from MSK-Leuk1 cells treated with vehicle or the indicated concentration of TGF-α (1-50 ng/mL) for 6 hours. In A and B, cellular lysate protein (100 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed sequentially for COX-2 and β-actin.
increased release of amphiregulin protein (Fig. 5A) occurred within 1 hour of TS exposure. This means that altered gene expression of amphiregulin cannot explain the rapid release of this ligand into the medium. In contrast to its effect on amphiregulin and TGF-α, TS did not affect levels of HB-EGF mRNA. In an effort to translate these in vitro findings to the clinical setting, we evaluated levels of amphiregulin and TGF-α mRNAs in the oral mucosa of active smokers versus never smokers. The amounts of both amphiregulin and TGF-α in oral mucosa were significantly greater in active smokers than in never smokers (Fig. 6). Finally, experiments were carried out to confirm that amphiregulin and TGF-α themselves induce COX-2. As shown in Fig. 7, treatment of MSK-Leuk1 cells with either exogenous amphiregulin or TGF-α resulted in dose-dependent induction of COX-2.

Discussion
In this study, we have shown that levels of COX-2 are increased in the oral mucosa of active smokers versus never smokers. Consistent with this finding and previous reports (43, 44), treatment with TS induced COX-2 in cell lines derived from the human aerodigestive tract. Several observations support a critical role for EGFR in TS-mediated induction of COX-2. Treatment with TS stimulated the phosphorylation of EGFR. Two different types of evidence have firmly established a causal link between TS-mediated activation of EGFR and the induction of COX-2. Treatment with AG1478, an inhibitor of EGFR tyrosine kinase activity, blocked the induction of COX-2 by TS. Moreover, antibody blockade of the ligand-binding site of EGFR abrogated TS-mediated induction of COX-2. To our knowledge, this study provides the first evidence that TS-mediated induction of COX-2 is EGFR dependent. Notably, these findings are consistent with previous evidence that activation of EGFR signaling can drive COX-2 transcription and PG biosynthesis (5, 45, 46).

Recently, tobacco smoke was found to stimulate the phosphorylation of EGFR in lung epithelial cells (47). This was a consequence of smoke-induced activation of metalloproteinase activity that, in turn, led to cleavage of transmembrane amphiregulin with shedding of active ligand (47). Subsequently, the released mature ligand stimulated EGFR tyrosine kinase activity by an autocrine or paracrine mechanism. Our finding that brief treatment with TS stimulated the release of amphiregulin protein before any change in amounts of amphiregulin mRNA was observed is consistent with this mechanism. Stimulation of G-protein-coupled receptors has been reported to cause metalloproteinase-mediated cleavage of proamphiregulin resulting in transactivation of EGFR (48). Hence, it will be of interest to determine whether G-protein-coupled receptors are involved in TS-mediated induction of COX-2.

In this study, we also showed that exposure of oral epithelial cells to tobacco smoke markedly induced mRNAs for amphiregulin and TGF-α. To our knowledge, this effect of tobacco smoke has not been previously reported. Consistent with these in vitro findings, increased amounts of amphiregulin and TGF-α mRNAs were also found in the oral mucosa of active smokers versus never smokers. This finding strongly suggests that the inductive effects of tobacco smoke observed in vitro translate to humans in vivo. Taking these data together, we postulate that tobacco smoke can stimulate both the synthesis and release of ligands of the EGFR. Given the importance of EGFR in driving mitogenesis, these results provide mechanistic insights that may help to explain the increase in cell proliferation observed in the aerodigestive tracts of active smokers (49, 50).

Cross-talk exists between EGFR and COX-2. As detailed above, activation of EGFR signaling leads to increased amounts of COX-2 and enhanced production of PGE2 (5, 45, 46). Importantly, there is also growing evidence that COX-2–derived PGs can activate EGFR signaling (51, 52). 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 also 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 (31, 32). 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 (53, 54) and activation of EGFR signaling or stimulation of PG biosynthesis enhances cell proliferation (24, 42, 55), which, in turn, should increase the mutagenicity of tobacco smoke. Selective inhibitors of COX-2 and EGFR are clinically available. This study provides a mechanism-based rationale for evaluating whether a selective inhibitor of COX-2 or EGFR tyrosine kinase used alone or in combination can prevent or delay the onset of tobacco-related malignancies of the aerodigestive tract.

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References
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