Tobacco Smoke Stimulates the Transcription of Amphiregulin in Human Oral Epithelial Cells: Evidence of a Cyclic AMP-Responsive Element Binding Protein–Dependent Mechanism

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Abstract

Activation of epidermal growth factor receptor (EGFR)-mediated signaling has been implicated in the pathogenesis of tobacco smoke–induced cancers. Recently, elevated levels of amphiregulin, a ligand of the EGFR, were found in the oral mucosa of smokers. The main objective of this study was to elucidate the mechanism by which tobacco smoke induces amphiregulin. Treatment of a nontumorigenic human oral epithelial cell line (MSK-Leuk1) with a saline extract of tobacco smoke stimulated amphiregulin (AR) transcription resulting in increased amounts of amphiregulin mRNA and protein. Tobacco smoke stimulated the cyclic AMP (cAMP) →protein kinase A (PKA) pathway leading to increased cAMP-responsive element binding protein–dependent activation of AR transcription. These inductive effects of tobacco smoke were dependent on the aryl hydrocarbon receptor (AhR). In fact, α-naphthoflavone, an AhR antagonist, blocked tobacco smoke–mediated induction of binding of cAMP-responsive element binding protein to the AR promoter and thereby suppressed the induction of amphiregulin. Notably, treatment of MSK-Leuk1 cells with tobacco smoke or exogenous amphiregulin stimulated DNA synthesis. An inhibitor of EGFR tyrosine kinase or a neutralizing antibody to amphiregulin abrogated the increase in DNA synthesis mediated by tobacco smoke. Taken together, these findings suggest that tobacco smoke stimulated a signaling pathway comprised of AhR→cAMP→PKA resulting in enhanced AR transcription and increased DNA synthesis. The ability of tobacco smoke to induce amphiregulin and thereby enhance DNA synthesis is likely to contribute to the procarcinogenic effects of tobacco smoke. (Cancer Res 2005; 65(13): 5982-8)

Introduction

Smoking is an important risk factor for multiple human malignancies including cancers of the aerodigestive tract (1, 2). Many individuals are unable to quit smoking. Hence, there is a significant unmet need for chemopreventive agents that protect against the carcinogenic effects of tobacco smoke. Treatment with β-carotene increased the risk of lung cancer in smokers, underscoring the need for new therapeutic strategies (3). The successful development of targeted chemopreventive therapies will depend on a detailed understanding of the mechanisms underlying tobacco smoke–induced carcinogenesis.

Conversion of tobacco smoke–induced DNA adducts to mutations can only occur in proliferating cells (4, 5). Increased cell proliferation has been observed in the aerodigestive tracts of smokers (6, 7). These findings raise the intriguing possibility that tobacco smoke amplifies its own mutagenicity by stimulating cell proliferation. Tobacco smoke can stimulate cell proliferation by activating the epidermal growth factor receptor (EGFR; refs. 8–10). Activation of EGFR occurs as a consequence of tobacco smoke–induced synthesis and release of ligands of the EGFR including amphiregulin (9, 10). Recently, elevated levels of amphiregulin were found in the oral mucosa of smokers (10). Taken together, it seems likely that increased expression of amphiregulin contributes to the increased cell proliferation observed in the aerodigestive tracts of smokers.

Levels of amphiregulin are commonly increased in human malignancies including those of the aerodigestive tract and correlate with poor prognosis (11, 12). Several studies have suggested that activation of the cyclic AMP (cAMP) →protein kinase A (PKA) pathway can induce the transcription of amphiregulin (AR), resulting in enhanced cell proliferation (13, 14). Little is known, however, about the mechanism by which tobacco smoke induces amphiregulin (15). In the present study, we first determined that tobacco smoke stimulated a signaling pathway comprised of aryl hydrocarbon receptor (AhR)→cAMP→PKA resulting in enhanced AR transcription. Subsequently, we showed that tobacco smoke–mediated induction of amphiregulin was responsible for increased DNA synthesis. These findings provide new insights into the procarcinogenic effects of tobacco smoke. Moreover, this study highlights the potential importance of EGFR as a molecular target for the chemoprevention of tobacco smoke–related malignancies of the human aerodigestive tract.

Materials and Methods

Materials. Keratinocyte basal and growth media were from Clonetics Corp. (San Diego, CA). H-89, 6-formylindolo[3,2-b]carbazole (FICZ), forskolin, and cAMP enzyme immunoassay kit were from Biomol (Plymouth Meeting, PA). α-Naphthoflavone and kits for lactate dehydrogenase were from Sigma Chemical Co. (St. Louis, MO). Amphiregulin, enzyme immunoassay kits for amphiregulin, normal immunoglobulin G (IgG), and anti-amphiregulin were obtained from R&D Systems, Inc. (Minneapolis, MN). AG1478 and PKA activity assay kits were from Calbiochem (San Diego, CA). Antibodies to human cAMP-responsive element binding protein (CREB), nuclear factor κB (NF-κB) p65, NF-κB p50, and PEA3 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). RNA was prepared using kits from Qiagen (Chatsworth, CA). [3H]Thymidine was from Perkin-Elmer Life Sciences (Boston, MA).
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Tissue culture. The MSK-Leuk1 cell line was established from a dysplastic leukoplakia lesion adjacent to a squamous cell carcinoma of the tongue (16). Cells were routinely maintained in keratinocyte growth medium supplemented with bovine pituitary extract. Cells were grown in basal medium for 24 hours before treatment. Treatment with vehicle (PBS) or a saline extract of tobacco smoke (see below) was carried out under serum-free conditions. Cellular cytotoxicity was assessed by measurements of cell number, trypan blue exclusion, and release of lactate dehydrogenase.

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 variables 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 was subjected to sterile conditions before premeasured amounts of sterile PBS (pH 7.4). This smoke in PBS represents whole trapped mainstream smoke abbreviated as tobacco smoke (10). Quantitation of smoke content is expressed in puffs per milliliter of PBS with one cigarette yielding about 8 puffs drawn into a 5 mL volume. The final concentration of tobacco smoke in the cell culture medium is expressed as puffs per milliliter of medium.

Measurements of amphiregulin. MSK-Leuk1 cells were plated in six-well dishes and grown to 60% confluence in growth medium. Following treatment, levels of amphiregulin protein released in the medium were quantified by enzyme immunoassay according to the instructions of the manufacturer. Amounts of amphiregulin released in the medium are expressed as picograms per microgram of cellular protein.

To determine cellular levels of mRNA for amphiregulin, total cellular RNA was isolated from cells according to the instructions of the manufacturer. Reverse transcription was done in a thermocycler (GeneAmp PCR System 2400, Perkin-Elmer, Norwalk, CT) using 2 μg of RNA per 40 μL reaction. The reaction mixture contained 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2.5 mmol/L MgCl2, 0.5 mmol/L deoxynucleotide triphosphate, 2.5 μmol/L oligo d(T)16 primer, 40 units RNase inhibitor, and 100 units murine leukemia virus reverse transcriptase (Roche Applied Science, Indianapolis, IN). Samples were amplified for 10 minutes at 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 5 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 30 seconds, 62°C for 30 seconds, and then 70°C for 45 seconds. Subsequently, extension was carried out at 70°C for 10 minutes. Primer sequences were as follows: amphiregulin, forward 5'-AAGCGTGAAACATTTTTACTG-C3', reverse 5'-TTTCTAGGACTT-TTCCCCAC-3'; β-actin, forward 5'-GGTCACCCACACGTGACCG-3', reverse 5'-GGATGCCACACAGACTCCTAG-C3'. PCR products were electrophoresed on a 1% agarose gel with 0.5 μg/mL ethidium bromide and photographed under UV light. The identity of each PCR product was confirmed by DNA sequencing. A computer densitometer (Chem Doc; Bio-Rad, Hercules, CA) was used to quantify the density of the different bands. Data are expressed in arbitrary units.

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. The AR promoter-luciferase constructs pGL2-A, pGL2-B, pGL2-ΔCRE, pGL2-C, and pGL2-ΔCRE containing the 5′-flanking region of the human AR gene have previously been described and were a generous gift of Dr. Sean Bong Lee (NIH, Bethesda, MD; ref. 17). For the cells in each well, 2 μg of plasmid DNA were introduced using 2 μg of Lipofectamine 2000 as per instructions of the manufacturer. After 6 hours of incubation, the medium was replaced with basal medium. The activities of luciferase and β-galactosidase were measured in cellular extracts as in previous studies (18).

Electrophoretic mobility shift assays. Cells were harvested and nuclear extracts were prepared. For binding studies, oligonucleotides containing the cAMP-responsive element of the AR promoter were synthesized (Sigma and Genosys, The Woodlands, TX): 5'-GGGCCCCCAGGCGCT-CATGGGCT-3' (sense) and 5'-AGCCCATGACGTCAAGGCCG-3' (antisense). Complementary oligonucleotides were annealed in 20 mmol/L Tris-HCl (pH 7.6), 50 mmol/L NaCl, 10 mmol/L MgCl2, and 1 mmol/L DTT. The annealed oligonucleotide was phosphorylated at the 5′-end with γ-32P-ATP and T4 polynucleotide kinase. The binding reaction was done by incubating 8 μg of nuclear protein in 10 mmol/L Tris-HCl (pH 7.5), 12% glycerol, and 50 μg/mL poly(deoxyinosinic-deoxyxystyridic acid) in a final volume of 10 μL for 10 minutes at 25°C. The labeled oligonucleotide was added to the reaction mixture and allowed to incubate for an additional 20 minutes. For supershift assays, antibody was added after incubation of the probe, nuclear extract, and binding buffer. The reaction mixture was then left at room temperature for 20 minutes. For competition assays, unlabeled competitor oligonucleotide was preincubated with nuclear extract and binding buffer for 15 minutes before the addition of the labeled probe. The binding reaction was then incubated on ice for an additional 15 minutes before electrophoresis. The samples were electrophoresed on a 4% nondenaturing polyacrylamide gel. The gel was then dried and submitted to autoradiography at ~80°C.

Measurements of cyclic AMP levels. Cells were plated at 5 × 10^4/well in six-well dishes and grown to 70% confluence before treatment. Amounts of cAMP were measured by enzyme immunoassay. Production of cAMP was normalized to protein concentration.

Protein kinase A activity. Cells were plated at 5 × 10^4/well in six-well dishes and grown to 70% confluence before treatment. PKA activity was measured according to the instructions of the manufacturer. PKA activity was normalized to protein concentration.

DNA synthesis assay. Incorporation of [3H]thymidine was used to measure DNA synthesis. Cells were plated at 1 × 10^4 cells/well in a 96-well plate and allowed to adhere overnight before being treated. Following treatment, [3H]thymidine (0.1 μCi/well) was added for 6 hours. Cells were then washed twice with PBS. Radioactivity was then measured with a Beckman LS6800 liquid scintillation counter (Beckman, Fullerton, CA).

Statistics. Comparisons between groups were made by the Student’s t test. A difference between groups of P < 0.05 was considered significant.

Results

Treatment with tobacco smoke induces the expression of amphiregulin. Treatment with tobacco smoke led to an ~3-fold increase in amounts of amphiregulin protein in the medium (Fig. 1A). To determine if this increase was due at least, in part, to altered expression, levels of amphiregulin mRNA were measured. As shown in Fig. 1B, tobacco smoke markedly induced levels of amphiregulin mRNA. Subsequently, transient transfections were carried out to elucidate the effects of tobacco smoke on AR promoter activity. Treatment with tobacco smoke led to nearly a doubling of AR promoter activity in MSK-Leuk1 cells transiently transfected with the reporter vector pGL2-A, which contains the AR promoter sequence from −850 to −87 (Fig. 2B). To define the region of the AR promoter (Fig. 2A) that responded to tobacco smoke, a series of human AR promoter deletion constructs was used. Cells were transfected with pGL2-B, which contains 136 nucleotides (−328 to −192) including the Wilms’ tumor suppressor WT1 responsive element, the cAMP-responsive element, and TATA box. Luciferase activity was increased by tobacco smoke compared with vehicle-treated cells. Mutation of the cAMP-responsive element site (pGL2-ΔCRE) abrogated the increase in AR promoter activity mediated by tobacco smoke. We confirmed the critical role of the cAMP-responsive element for tobacco smoke–mediated activation of AR transcription using pGL2-C and pGL2-ΔCRE reporter vectors, which contain only 83 nucleotides (−275 to −192), including the cAMP-responsive element and TATA box.
More specifically, mutation of the cAMP-responsive element resulted in a loss of tobacco smoke–mediated activation of the AR promoter (Fig. 2B).

Electrophoretic mobility shift assays were done to identify the transcription factor that mediated the induction of amphiregulin by tobacco smoke. Tobacco smoke caused increased binding to the cAMP-responsive element site of the AR promoter (Fig. 3). The increase in binding to the AR cAMP-responsive element was competed by incubating nuclear extract from tobacco smoke–treated cells with an excess of cAMP-responsive element cold probe. Supershift analyses identified CREB in the binding complex. A supershift was not observed with antibodies to NF-kB p50 or p65 or PEA3 (Fig. 3). These findings are consistent with prior evidence that CREB can stimulate AR transcription (13).

Role of the cyclic AMP–protein kinase A pathway in tobacco smoke–mediated induction of amphiregulin. Previous studies have found that activation of the cAMP–PKA pathway stimulates CREB-dependent activation of amphiregulin transcription (13, 14). Given this background, we investigated the effects of tobacco smoke on levels of cAMP and PKA activity. A rapid increase in levels of cAMP and PKA activity was observed following treatment with tobacco smoke (Fig. 4A and B). Forskolin, a known inducer of adenylate cyclase activity, served as a positive control and had similar stimulatory effects in these experiments. It was next important to confirm that activation of the cAMP–PKA pathway was causally linked to the induction of amphiregulin. To address this question, we used H89, a selective inhibitor of PKA. As shown in Fig. 4C, treatment with H-89 abrogated the induction of amphiregulin mediated by tobacco smoke.

The aryl hydrocarbon receptor is important for induction of amphiregulin by tobacco smoke. The AhR is activated by polycyclic aromatic hydrocarbons in tobacco smoke. Previous studies have suggested that the AhR mediates some of the procarcinogenic effects of tobacco smoke (1). Experiments were next done to determine whether this receptor was involved in mediating the stimulatory effects of tobacco smoke on AR transcription. In the first experiment (Fig. 5A), we evaluated whether FICZ, a prototypic AhR ligand, could induce amphiregulin. FICZ caused a rapid and marked increase in levels of amphiregulin. Next α-naphthoflavone, an AhR antagonist, was

**Figure 1.** Tobacco smoke induces the expression of amphiregulin. Cells were treated with vehicle or tobacco smoke (0.030 puffs/mL) for up to 16 hours. A, amounts of amphiregulin (AR) 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, reverse transcription-PCR (RT-PCR) was done at different time points for amphiregulin and β-actin mRNAs. Std, standard. Levels of amphiregulin mRNA are expressed in arbitrary units (AU).

**Figure 2.** The cAMP-responsive element is responsible for tobacco smoke–mediated activation of the amphiregulin promoter. A, structure of the AR promoter including deletion constructs. B, cells were transfected with 1.8 μg of a series of human AR promoter-luciferase constructs and 0.2 μg of pSVβ-gal. Following transfection, cells were treated with vehicle or tobacco smoke (TS; 0.03 puffs/mL). Reporter activities were measured in cellular extracts 6 hours after treatment. Luciferase activity represents data that have been normalized to β-galactosidase activity. Percent of vehicle-treated control. Columns, means (n = 6); bars, SD. *, P < 0.05; **, P < 0.01, compared with vehicle-treated control.

**Figure 3.** Increased binding of CREB to the cAMP-responsive element of the AR promoter is detected in tobacco smoke–treated cells. Cells were treated with vehicle (lane 1) or tobacco smoke (0.03 puffs/mL; lanes 2-9) for 4 hours. Eight micrograms of nuclear protein were incubated with a 32P-labeled oligonucleotide containing the cAMP-responsive element of AR. Lanes 3 to 7, nuclear protein incubated with antibodies to CREB (3 and 4), NF-kB p65 (5), NF-kB p50 (6) or PEA3 (7). Lane 9, IgG. Lane 8, nuclear protein incubated with a 100-fold excess of unlabeled oligonucleotide containing the cAMP-responsive element of amphiregulin. The protein-DNA complex that formed was separated on 4% polyacrylamide gel.
Remarkably, α-naphthoflavone abrogated the induction of amphiregulin by tobacco smoke (Fig. 5B). Additional experiments were carried out to evaluate whether the effects of tobacco smoke on the AhR were proximal to activation of cAMP–PKA signaling. Notably, treatment with α-naphthoflavone blocked the increase in levels of cAMP and PKA activity mediated by tobacco smoke (Fig. 5C and D). Taken together, these results suggest that tobacco smoke–mediated activation of the cAMP–PKA pathway is AhR dependent. To further investigate this question, we investigated whether α-naphthoflavone suppressed tobacco smoke–induced binding of CREB to the cAMP-responsive element of the AR promoter. As shown in Fig. 6, treatment with tobacco smoke augmented binding of nuclear protein to the cAMP-responsive element, an effect that was suppressed by α-naphthoflavone, an AhR antagonist.

Amphiregulin stimulates DNA synthesis. Finally, we investigated the role of amphiregulin in stimulating DNA synthesis. As shown in Fig. 7A, treatment of MSK-Leuk1 cells with tobacco smoke led to a significant increase in DNA synthesis, an effect that was blocked by AG1478, an inhibitor of EGFR tyrosine kinase. Importantly, the inductive effect of tobacco smoke on DNA synthesis was also abrogated by the addition of an antibody to

![Figure 4](image-1.png)

**Figure 4.** Tobacco smoke induces amphiregulin by stimulating the cAMP–PKA pathway. A and B, cells were treated with tobacco smoke (0.03 puffs/mL) for 0 to 60 minutes. Forskolin is known to stimulate adenylate cyclase resulting in activation of the cAMP–PKA pathway. Hence, cells were treated with 10 μmol/L forskolin for 10 minutes as a positive control. Cellular levels of cAMP (A) and PKA activity (B) were determined at each time point. Columns, means (n = 6); bars, SD. *, P < 0.05; **, P < 0.01, compared with time zero. C, cells were pretreated with vehicle or H-89 (5 μmol/L), a PKA inhibitor, for 2 hours. Subsequently, cells were treated with vehicle or tobacco smoke (0.03 puffs/mL) for 4 or 8 hours. RT-PCR was done for amphiregulin and β-actin mRNAs.

![Figure 5](image-2.png)

**Figure 5.** AhR is important for tobacco smoke–mediated induction of amphiregulin. A, treatment with FICZ (1 μmol/L), an AhR agonist, caused a rapid increase in amounts of amphiregulin. B, cells were pretreated with vehicle or α-naphthoflavone (α-NF), an AhR antagonist, for 6 hours. Subsequently, cells were treated with vehicle or tobacco smoke (0.03 puffs/mL) for 4 hours. A and B, RT-PCR was done for amphiregulin and β-actin mRNAs. Levels of amphiregulin mRNA are expressed in arbitrary units. C and D, cells were pretreated with vehicle or α-naphthoflavone for 6 hours before receiving vehicle or tobacco smoke (0.03 puffs/mL) for 30 minutes. Cellular levels of cAMP (C) and PKA activity (D) were determined. Columns, means (n = 6); bars, SD. *, P < 0.05; **, P < 0.01, compared with tobacco smoke–treated cells.
amphiregulin (Fig. 7B). In contrast, control IgG had no effect on the increase in DNA synthesis mediated by tobacco smoke. Finally, treatment with exogenous amphiregulin led to an increase in DNA synthesis that was comparable to tobacco smoke (Fig. 7C). Taken together, these findings strongly suggest that the inductive effect of tobacco smoke on DNA synthesis is mediated by amphiregulin.

Discussion

In the present experiments, we have shown that tobacco smoke induced AR transcription. To more completely understand this effect, we identified the AR promoter element that mediated the inductive effect of tobacco smoke. The results of transient transfections and electrophoretic mobility shift assays suggested that tobacco smoke activated AR transcription by enhancing the binding of CREB to the cAMP-responsive element of the AR promoter. This finding is consistent with at least two previous studies that showed the importance of the cAMP-responsive element for the regulation of AR gene expression (13, 14). To our knowledge, this is the first time that tobacco smoke has been shown to stimulate CREB-dependent gene expression. Based on this finding, it will be worthwhile to determine whether the expression of other known CREB-dependent genes (e.g., c-fos) is altered by tobacco smoke.

A variety of stimuli regulate transcription via CREB by causing an elevation of the second messenger cAMP, which activates PKA. PKA phosphorylates CREB, which, in turn, binds to cAMP-responsive elements in the promoters of many cAMP-regulated genes (19). Given this background and our finding that tobacco smoke enhanced AR transcription by a CREB-dependent mechanism, we investigated whether tobacco smoke stimulated the cAMP—PKA pathway. Remarkably, exposure to tobacco smoke led to a rapid increase in cAMP levels and PKA activity. Importantly, an inhibitor of PKA activity blocked tobacco smoke—mediated induction of amphiregulin. This finding enabled us to conclude that tobacco smoke—mediated activation of the cAMP—PKA pathway was causally linked to the induction of amphiregulin.

Tobacco smoke contains ~4,000 compounds (1). More than 100 carcinogens, mutagens, and tumor promoters have been identified in tobacco smoke. The polycyclic aromatic hydrocarbons are among the best characterized carcinogens in smoke. Polycyclic aromatic hydrocarbons such as benzo[a]pyrene bind to and activate the AhR, leading to changes in both cell signaling and gene transcription (20–23). Notably, AhR has been linked to polycyclic aromatic hydrocarbon—induced carcinogenesis (24).
Previous studies have shown that compounds that bind to and activate the AhR, such as dioxin, induce the expression of EGFR ligands (25, 26). Therefore, we investigated whether the AhR was involved in tobacco smoke-mediated induction of AR transcription. Several observations support a critical role for the AhR in tobacco smoke–mediated induction of amphiregulin. Treatment with FICZ, an AhR agonist, markedly induced amphiregulin thereby recapitulating the effects of tobacco smoke. Moreover, α-naphthoflavone, an AhR antagonist, blocked tobacco smoke–mediated induction of amphiregulin. Finally, tobacco smoke–mediated induction of CREB binding to the AR promoter was attenuated by treatment with α-naphthoflavone. It was next important to determine whether tobacco smoke–mediated activation of the AhR was proximal to stimulation of cAMP→PKA signaling. In support of this notion, we showed that α-naphthoflavone blocked tobacco smoke–mediated activation of cAMP→PKA signaling. Thus, it seems that tobacco smoke activates a signaling pathway comprised of AhR→cAMP→PKA resulting in enhanced AR transcription. Additional studies will be needed to define the precise mechanism by which ligands of the AhR activate cAMP→PKA signaling.

In addition to stimulating AR transcription, tobacco smoke can enhance metalloproteinase activity (9, 10). This leads, in turn, to increased cleavage of transmembrane amphiregulin with shedding of active ligand. Several studies have shown that amphiregulin, a ligand of the EGFR, can drive cell proliferation (27–31). This is consistent with extensive evidence that activation of EGFR signaling drives mitogenesis (32, 33). Hence, it was important to evaluate the functional consequences of enhanced release of amphiregulin. Several findings firmly established a causal link between tobacco smoke–mediated induction of amphiregulin and increased DNA synthesis. Treatment with AG178, an inhibitor of EGFR tyrosine kinase, or anti-amphiregulin abrogated tobacco smoke–mediated induction of DNA synthesis. Moreover, treatment of cells with exogenous amphiregulin induced DNA synthesis. Taking these data together, we postulate that tobacco smoke induces the synthesis and release of amphiregulin, resulting in activation of EGFR signaling and thereby enhanced mitogenesis. These results provide mechanistic insights that help explain the increase in cell proliferation observed in the aerodigestive tracts of smokers (6, 7). It should be noted that other components of tobacco smoke might also contribute to changes in cell proliferation. For example, nicotine can stimulate cell proliferation (34, 35). In all likelihood, multiple constituents of tobacco smoke contribute to the increase in cell proliferation observed in vivo.

In summary, we showed that tobacco smoke stimulated a signaling pathway comprised of AhR→cAMP→PKA resulting in enhanced AR transcription and increased DNA synthesis. Because conversion of DNA adducts to mutations occurs in proliferating cells (4, 5), it is reasonable to postulate that tobacco smoke–mediated induction of amphiregulin will amplify the effect of a given dose of tobacco smoke on tumor initiation. Inhibitors of EGFR tyrosine kinase are clinically available (36, 37). This study strengthens the rationale for evaluating whether an inhibitor of EGFR tyrosine kinase can prevent or delay the onset of tobacco smoke–related malignancies of the aerodigestive tract.

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References


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