NEOPLASIA
www.neoplasia.com

Volume 15 Number 4 April 2013 pp. 454–460 454

Adenomatous Polyposis Coli–Mediated Accumulation of Abasic DNA Lesions Lead to Cigarette Smoke Condensate–Induced Neoplastic Transformation of Normal Breast Epithelial Cells[1]

Aruna S. Jaiswal[*,†], Harekrushna Panda[*], Christine A. Pampo[‡], Dietmar W. Siemann[‡], C. Gary Gairola[§], Robert Hromas[†] and Satya Narayan[*]

*Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL; [†]Department of Medicine, University of Florida, Gainesville, FL; [‡]Department of Radiation Oncology, University of Florida, Gainesville, FL; [§]Graduate Center for Toxicology, College of Medicine, University of Kentucky, Lexington, KY

Abstract

Adenomatous polyposis coli (APC) is a multifunctional protein having diverse cellular functions including cell migration, cell-cell adhesion, cell cycle control, chromosomal segregation, and apoptosis. Recently, we found a new role of APC in base excision repair (BER) and showed that it interacts with DNA polymerase β and 5′-flap endonuclease 1 and interferes in BER. Previously, we have also reported that cigarette smoke condensate (CSC) increases expression of APC and enhances the growth of normal human breast epithelial (MCF10A) cells *in vitro*. In the present study, using APC overexpression and knockdown systems, we have examined the molecular mechanisms by which CSC and its major component, Benzo[*α*]pyrene, enhances APC-mediated accumulation of abasic DNA lesions, which is cytotoxic and mutagenic in nature, leading to enhanced neoplastic transformation of MCF10A cells in an orthotopic xenograft model.

Neoplasia (2013) 15, 454–460

Introduction

It is well accepted that cigarette smoke is a carcinogen (WHO, 2004) and linked as a risk factor for cancer development in many organs, including lung [1,2], head and neck [3], kidney [4,5], bladder [6], pancreas [7], and colon [8]. However, a definitive link of cigarette smoking with breast cancer development is not well established. In the past several years, many epidemiological studies supported the association of cigarette smoking and breast cancer risk. However, there are also reports suggesting that cigarette smoking has little or no independent effect on breast cancer risk. Recently, a Canadian Experts Panel on tobacco smoke and breast cancer risk extensively reviewed the research findings in this area and concluded that the association between active smoking and breast cancer is consistent with causality. In addition, the association between secondhand smoking and breast cancer among younger, primarily premenopausal women who have never smoked was consistent with causality [9]. These conclusions were further supported by other recent findings [10–12].

Tobacco smoke contains more than 170 toxic substances, including 33 hazardous pollutants, 47 chemicals restricted as hazardous waste, 3 regulated air pollutants, and 67 known human or animal carcinogens [13]. Twenty of these carcinogens, including polycyclic aromatic hydrocarbon, benzo[α]pyrene (B[α]P), have been demonstrated to induce mammary tumors in animal models [14]. Laboratory studies indicate that secondhand smoke is three to four times more toxic than mainstream smoke [15]. The biologic possibility of cigarette smoking and breast cancer is supported by the smoking-specific DNA adducts and mutations in the *p53* gene in the breast

Abbreviations: APC, adenomatous polyposis coli; AP, apurinic/apyrimidinic; B[α]P, benzo[α]pyrene; CSC, cigarette smoke condensate; Fen1, 5′-flap endonuclease 1; LP-BER, long-patch base excision repair; SP-BER, short-patch base excision repair; Pol-β, DNA polymerase β

Address all correspondence to: Dr Satya Narayan, Department of Anatomy and Cell Biology, Basic Science Building, Room B1-016, 1333 Center Drive, Gainesville, FL 32610. E-mail: snarayan@ufl.edu

[1]The financial support for this study was provided by Flight Attendant Medical Research Institute (Miami, FL) to S.N. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.
Received 9 January 2013; Revised 6 February 2013; Accepted 8 February 2013

DOI 10.1593/neo.13176

tissues of smokers [16–20]. In recent studies, we showed that cigarette smoke condensate (CSC), a surrogate of cigarette smoke, induces expression of adenomatous polyposis coli (APC) levels, blocks base excision repair (BER), and causes transformation of normal breast epithelial cells *in vitro* [21,22]. The decreased BER is expected to increase apurinic/apyrimidinic (AP) lesions. Whether CSC-induced APC levels are linked with the accumulation of AP lesions and neoplastic transformation of normal breast epithelial cells is not known. In the present study by using an orthotopic xenograft model, we have shown a link of CSC treatment, APC levels, and accumulation of AP lesions with CSC-induced breast carcinogenesis.

Materials and Methods

Maintenance of Cells and Treatment

The spontaneously immortalized human normal breast epithelial cell line MCF10A was grown at 37°C under a humidified atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium/F-12 medium supplemented with 5% heat inactivated horse serum (Sigma Chemical Co, St Louis, MO), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.5 μg/ml hydrocortisone, 100 ng/ml cholera toxin, 10 μg /ml insulin, 10 ng/ml epidermal growth factor, and 1% (wt/vol) of L-glutamine. After cells reached 60% confluence, they were treated with CSC or B[α]P as shown in the figure legends.

Preparation of CSC

CSC was prepared from the University of Kentucky Reference Cigarette 1R4F (9 mg of tar and 0.8 mg of nicotine/cigarette) adapting the previously described procedure [23]. Briefly, the "tar" or particulate phase of smoke was collected on a Cambridge filter pad from cigarettes smoked under standard Federal Trade Commission conditions (35-ml puff volume of a 2-second duration) [24] and dissolved in DMSO at 40 mg/ml, aliquoted into small vials, and stored frozen at −80°C. On the day of the experiment, each vial of CSC solution was opened and diluted in the cell culture medium to obtain the desired concentration, vortexed vigorously, and used for the treatment of cells. Control cells were treated with medium containing an equivalent amount of DMSO.

Western Blot Analysis

For Western blot analysis, single-cell suspension of MCF10A cells were plated (2×10^6 cells per well) in triplicates on six-well plates. Once the cells were attached to the plates, they were transfected with 1 μg/ml pCMV-APC overexpression or pShRNA-APC knockdown plasmids for 18 hours. After 18-hour post-transfection, one group of cells were treated with 25 μg/ml or 50 μg/ml CSC or 25 μM or 50 μM B[α]P for additional 30 hours. Changes in protein levels subsequent to the treatment of MCF10A cells with CSC or B[α]P was determined by Western blot analysis using whole-cell extracts, as described previously [25]. The antibodies used to detect levels of APC and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Oncogene Research Products (Cambridge, MA) and Sigma Chemical Co, respectively.

Estimation of the Number of AP Lesions in Genomic DNA

For the number of AP lesion estimation, single-cell suspension of MCF10A cells were plated (2×10^6 cells per well) in triplicates on six-well plates. Once the cells are attached to the bottom of the plate, they were transfected with 1 μg/ml pCMV-APC overexpression or pShRNA-APC plasmids for 18 hours. After 18-hour post-transfection in one group, cells were treated with 25 μg/ml or 50 μg/ml CSC, while cells in the other group were treated with 25 μM or 50 μM B[α]P for additional 30 hours. For each group, appropriately control plasmids were transfected into MCF10A cells and treated with CSC or B[α]P. After treatment, cells were harvested and the AP lesion assay was performed with some modification as described in previous studies [26]. Briefly, DNA from different cells (5×10^6) was isolated using

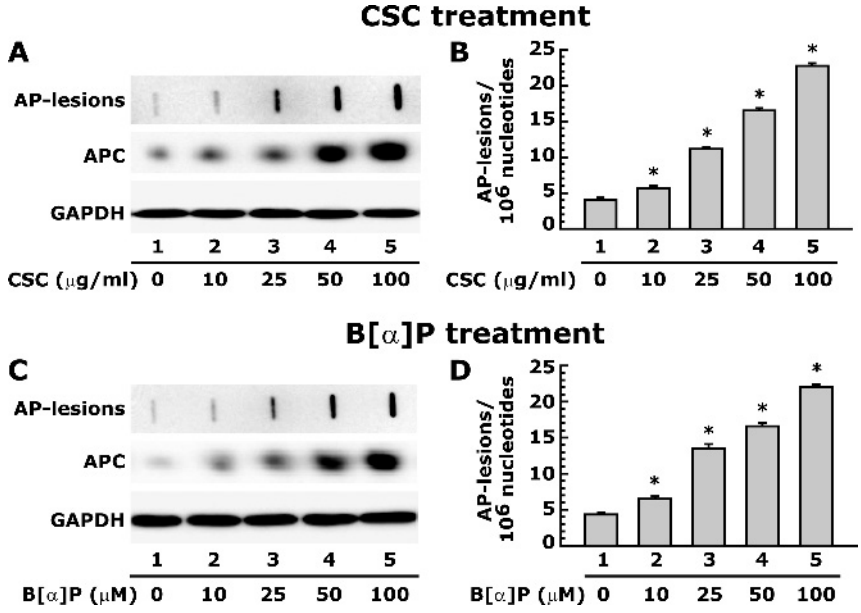

Figure 1. Effect of CSC and B[α]P treatments on the APC protein levels and the number of AP lesions in MCF10A cells. (A and C) AP lesion estimation and Western blot analysis of APC after treatment with CSC and B[α]P, respectively, for 30 hours. The protein loading is normalized with GAPDH. (B and D) Quantitative analysis of AP lesions. Data are means ± SE of three different determinations. *Significantly different than the untreated control group ($P < .05$).

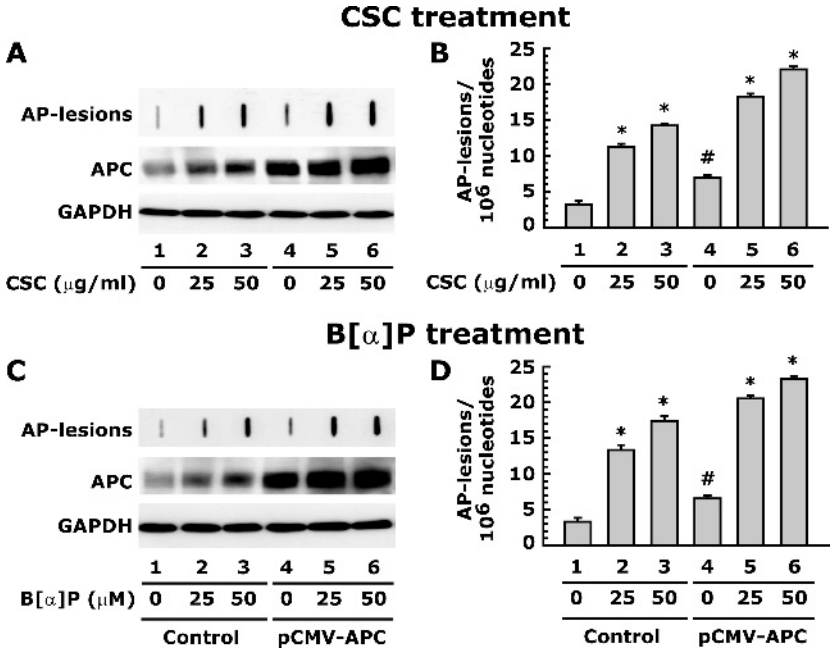

Figure 2. Effect of overexpression of APC on accumulation of AP lesions in MCF10A cells treated with CSC and B[*α*]P treatments. (A and C) AP lesion estimation and Western blot analysis of APC after transient transfection of pCMV-APC for 18 hours and then treatment with CSC and B[*α*]P, respectively, for additional 30 hours. The protein loading is normalized with GAPDH. (B and D) Quantitative analysis of AP lesions. Data are means ± SE of three different determinations. *Significantly different than the untreated control group ($P < .05$). #Significantly different than the control *versus* pCMV-APC–transfected group ($P < .05$).

GenElute Mammalian Genomic DNA Kit (Sigma-Aldrich, St Louis, MO). Five to 10 μg of the DNA in 150 μl of 1× phosphate-buffered saline was incubated with 1 mM aldehyde reactive probe (Cayman Chemicals, Ann Arbor, MI) at 37°C for 10 minutes, then ethanol precipitated and finally dissolved in 1× TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.2) and quantified. One microgram of the heat denatured DNA was slot blotted onto a positively charged nylon membrane (Amersham Corp, Piscataway, NJ). The nylon membrane was soaked with 5× SSC (0.75 M NaCl, 0.075 M trisodium citrate) at 37°C for 15 minutes, briefly air-dried, and baked in a vacuum oven at 80°C for 1 to 2 hours. The membrane was preincubated with 10 ml of Tris-NaCl buffer containing BSA [20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 0.5% casein, 0.25% BSA, and 0.1% Tween 20] at room temperature for 1 hour. The membrane was then incubated in the same solution containing streptavidin-conjugated HRP (BioGenex, San Ramon, CA) at room temperature for 30 to 45 minutes. The membrane was rinsed thrice for 10 minutes each with washing buffer (0.26 M NaCl, 1 mM EDTA, 20 mM Tris-HCl, and 0.1% Tween 20, pH 7.5), and the enzymatic activity on the membrane was visualized by the ECL Reagent (Amersham Corp). The membrane was then exposed to X-ray film (XAR 5×; Kodak, Rochester, NY) for 5 to 10 seconds. The developed film was analyzed using an Ultrascan XL scanning densitometer (Pharmacia, Uppsala, Sweden) and Gel Scan XL software (Pharmacia). The quantification was based on comparisons to internal standard DNA containing a known amount of AP lesions. All experiments were performed in triplicates.

Anchorage-independent Assay

The anchorage-independent growth of MCF10A cells was determined by colony formation efficiency in soft agar. Approximately 2000 MCF10A cells either transfected with pCMV-APC or pShRNA-APC plasmids were treated with CSC or B[*α*]P for 30 hours. After treatment, cells were mixed at 37°C with 2 ml of 0.33% (wt/vol) soft agar (Sigma Chemical Co) and then poured onto a layer of previously set 1.5 ml of 0.9% soft agar (wt/vol) in six-well tissue culture plates. The soft agar suspensions were prepared in complete Dulbecco's modified Eagle's medium/F-12 medium for MCF10A cells, as described above for the maintenance of cells. Cells were incubated for 4 weeks, then the growth of colonies was observed under an inverted microscope (Zeiss Axioplan-2 Imaging, Thornwood, NY) at ×20 magnification.

Cell Proliferation Assay

We selected few clones from soft agar plates and established them in culture. After three passages, we determined the transformation capacity of control (MCF10A) and treated (MCF10A-CSC and MCF10A-BP) cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (ATCC, Manassas, VA). In principle, the viable cell number is directly proportional to the purple formazan of the reduced MTT dye, which can be quantitatively measured by spectrophotometry. Briefly, 500 cells were plated onto quadruplets in 96-well flat-bottom tissue culture plates. After 12, 24, 48, 60, or 72 hours of incubation, 10 μl of MTT reagent was added to each well and incubated at 37°C for 4 hours to allow the formation of purple crystals of formazan. In total, 100 μl of detergent solution was added to each well, and the reaction mixture was incubated in dark for 2 to 4 hours (or sometimes overnight) at room temperature. The developed color density was then measured spectrophotometrically at 570 nm using the microplate reader (Vmax Kinetic Microplate Reader from Molecular Device, Sunnyvale, CA).

Orthotopic Tumor Growth and Histologic Studies

Next, to determine whether CSC- and B[α]P-induced clonogenic growth of MCF10A cells in soft agar have acquired true transformation capacity and whether it is linked with APC expression level, we performed *in vivo* experiments. We knocked down APC expression in MCF10A cells with pShRNA-APC plasmid, as we described in our previous studies [27]. In these studies, we used 4- to 6-week-old athymic (*nu/nu*) female mice from Harlan Laboratories (Indianapolis, Indiana). We orthotopically injected MCF10A and MCF10A-APC (KD) cells (10^6), which were either untreated or treated with 10 μg/ml CSC or 10 μM B[α]P for 30 hours. Cells were suspended in 100 μl of 50% Matrigel SQ basement membrane matrix (13.35 mg/ml; BD Biosciences, Bedford, MA) and injected in the fourth mammary fat pad on the right side of each mouse. The tumor growth was measured weekly by using digital calipers. After 4 months, tumor nodules were removed and fixed in 4% neutral buffered formalin for histologic evaluation with 5-μm sections stained with hematoxylin and eosin (H&E).

Statistical Analysis

All experiments were repeated at least three time and data were expressed as means ± SE. Statistical analysis was performed using Student's *t* test. The criterion for statistical significance was $P < .05$. For Western blot analysis data, band intensities were measured using ImageJ and normalized with GAPDH.

Results

CSC and B[α]P Treatments Cause Accumulation of AP Lesions in Normal Breast Epithelial Cells

First, we established whether the increased level of APC is linked with the accumulation of AP lesions after treatment with CSC and B[α]P. Results showed an increased level of APC in MCF10A cells in a dose-dependent manner after treatment with both CSC and B[α]P (Figure 1, *A* and *C*, respectively). To test our hypothesis that the increased level of APC blocks BER and causes accumulation of AP lesions, we determined the number of AP lesions in control and treated MCF10A cells. The AP lesions increased with the increase of APC levels after treatment with CSC and B[α]P (Figure 1, *B* and *D*, respectively). Thus, the results were consistent with our hypothesis.

Overexpression of APC in MCF10A Cells Further Increases the Number of AP Lesions after CSC and B[α]P Treatments

In these experiments, we further determined whether the increased level of APC is linked with increased number of AP lesions. We transfected MCF10A cells with plasmids carrying either the empty or the pCMV-APC overexpression plasmids as described above. After 18 hours, which is sufficient to see the transient overexpression of APC, cells were treated with different concentration of CSC (0, 25, and 50 μg/ml) or B[α]P (0, 25, and 50 μM) for additional 30 hours. Results showed that the treatment with CSC or B[α]P consistently increased the level of APC and AP lesions as compared with control (Figure 2, compare lane 1 with lane 4, respectively), which were further increased in APC-overexpressed cells (Figure 2, compare lanes 1 to 3 with lanes 4 to 6, respectively).

Down-regulation of APC in MCF10A Cells Decreases the Number of AP Lesions after CSC and B[α]P Treatments

To further test our hypothesis whether the increased level of APC is associated with the increased level of AP lesions, we knocked down APC levels in MCF10A cells by using ShRNA technique. We transfected cells with either pShRNA-APC (pShRNA-APC) or pShRNA-APCmut plasmids for 18 hours and then treated with different concentrations of

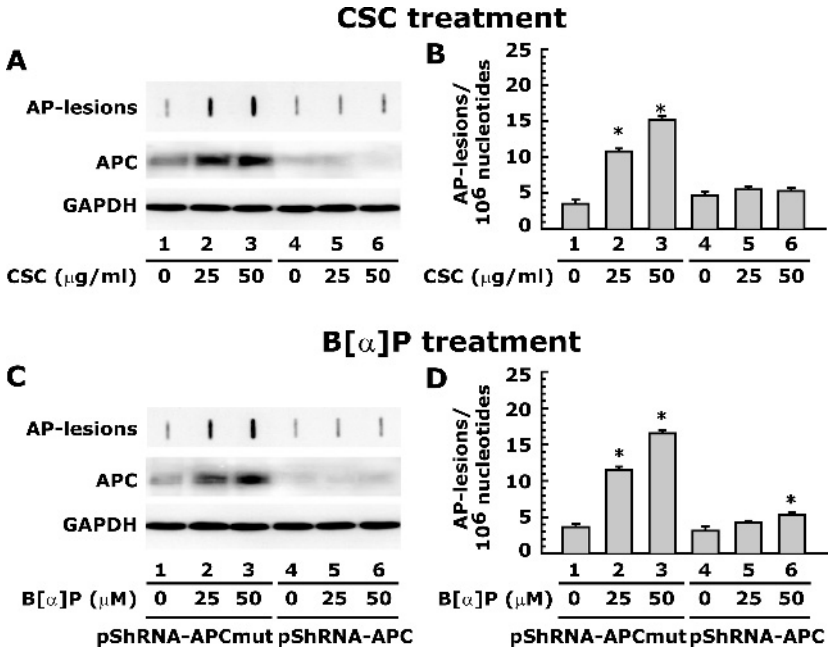

Figure 3. Correlation of down-regulation of APC with the accumulation of AP lesions in MCF10A cells treated with CSC and B[α]P treatments. (A and C) AP lesion estimation and Western blot analysis of APC after transient transfection of pShRNA-APCmut (lanes 1–3) or pShRNA-APC (lanes 4–6) for 18 hours and then treatment with CSC and B[α]P, respectively, for additional 30 hours. The protein loading is normalized with GAPDH. (B and D) Quantitative analysis of AP lesions. Data are means ± SE of three different determinations. *Significantly different than the untreated control group ($P < .05$).

CSC or B[α]P for additional 30 hours. Results showed that the knockdown of APC effectively decreased the number of AP lesions in MCF10A cells after treatment with CSC and B[α]P (Figure 3, compare lanes 1 to 3 and lanes 4 to 6, respectively). These results suggest that the absence of APC enables BER system to efficiently repair AP lesions in MCF10A cells treated with CSC and B[α]P.

Increased APC Level Is Associated with Increased Neoplastic Transformation of MCF10A Cells Treated with CSC and B[α]P—In Vitro *Studies*

To establish that the increased level of APC causes the accumulation of AP lesions, which, if not repaired efficiently, can result to neoplastic transformation of normal breast epithelial cells, we treated MCF10A cells with CSC or B[α]P, which were either overexpressed with pCMV-APC or knockdown with pShRNA-APC. After 30 hours of treatment, we first performed the anchorage-independent cell transformation assay. Results showed an increased number of transformed colonies in both CSC and B[α]P-treated groups (Figure 4, *A* and *B*, compare lane 1 with lane 2, respectively). Untreated MCF10A cells did not form any colony. These results are consistent with our previous findings [21]. Once the APC was overexpressed, the number of transformed colonies were further increased after CSC and B[α]P treatments (Figure 4, *A* and *B*, compare lane 2 with lane 4, respectively), which then decreased after the APC level was knocked down with pShRNA-APC (Figure 4, *A* and *B*, compare lane 4 with lane 6, respectively). Furthermore, when cells were transfected with pShRNA-APCmut, then the number of transformed colonies after CSC and B[α]P treatments increased compared to the one transfected with pShRNA-APC plasmid (Figure 4, *A* and *B*, compare lane 8 with lane 6, respectively). Thus, the increased level of APC suggests a link with increased number of soft agar colonies of MCF10A cells treated with CSC or B[α]P.

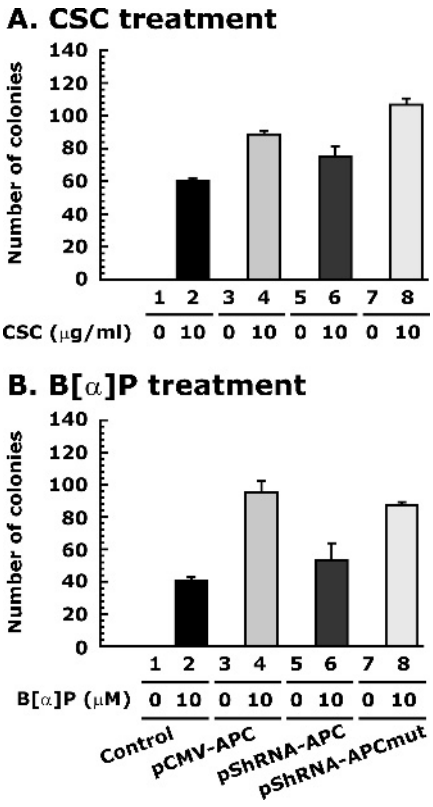

Figure 4. Correlation of APC levels and AP lesions with neoplastic transformation of MCF10A cells treated with CSC and B[α]P. (A and B) Number of MCF10A-transformed colonies after CSC or B[α]P treatment, respectively. Cells were transfected with empty vector, pCMV-APC, pShRNA-APCmut, or pShRNA-APC plasmids as indicated in the figure. Data are means ± SE of three different determinations.

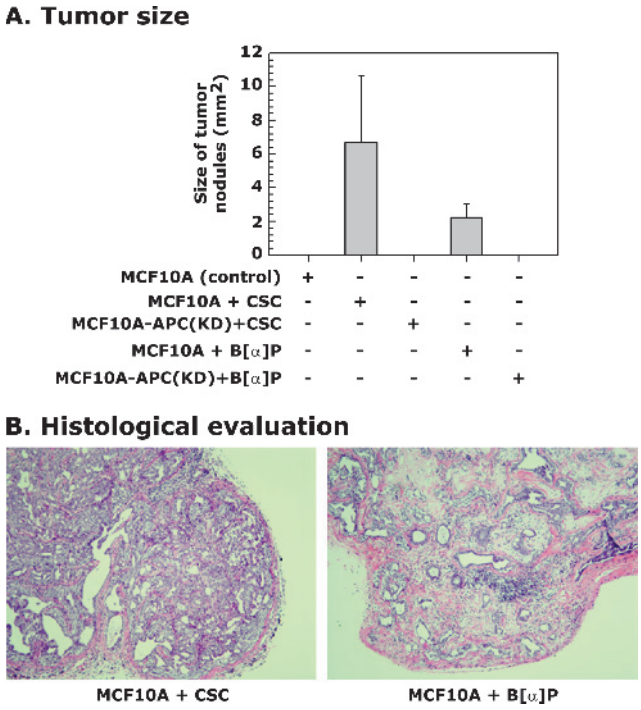

Figure 5. Histologic analysis of tumors produced by MCF10A cells after transformation with CSC and B[α]P treatments. (A) Size of tumor nodules measured at the 120th day after orthotopic xenograft implantation. (B) Histologic analysis of MCF10A-CSC and MCF10A-BP tumors after hematoxylin and eosin staining. Data are means ± SE of six animals in each group.

Increased APC Level Is Associated with Increased Transformation of MCF10A Cells Treated with CSC and B[α]P—In Vivo *Studies*

Next, to determine whether CSC- and B[α]P-induced clonogenic growth of MCF10A cells in soft agar have acquired true transformation capacity and whether it is linked with APC expression level, we performed *in vivo* tumorigenicity experiments. For these experiments, we orthotopically injected MCF10A and MCF10A-APC(KD) cells (APC-knockdown cells with pShRNA-APC) into mammary pads of female nude mice. Cells were either untreated or treated with 10 μg/ml CSC or 10 μM B[α]P for 30 hours. Results showed that the control MCF10A cells did not form any tumor, suggesting that they possessed the normal epithelial cell characteristics [28]. MCF10A cells that were treated with either CSC or B[α]P formed tumors. However, the MCF10A cells in which the APC was knocked down did not show any tumor formation (Figure 5*A*). In histologic analysis, MCF10A-CSC and MCF10A-BP tumors showed densely packed pleomorphic epithelial cells with numerous mitotic bodies and scattered ductular or acinar structures. The tumors also show neovascularity in the peritumoral stroma (Figure 5*B*). These results indicate a tumorigenic potential of MCF10A cells after treatment with CSC, which is higher

than the treatment with B[α]P. Furthermore, these results also suggest that the expression of APC is necessary for the transformation of MCF10A cells to maintain the tumorigenic capacity after CSC and B[α]P treatments.

Discussion

Results of the present study clearly provide evidence that CSC and B[α]P treatments induce AP lesions in MCF10A cells, which are linked with increased levels of APC. Cigarette smoke carcinogens can be activated into electrophilic intermediates by enzymes active in the human breast epithelial cells and the electrophilic metabolites of tobacco carcinogens may bind to DNA and form DNA adducts. There is evidence from the ^{32}P-postlabeling studies that smoking-related adducts are present in the breast tissue DNA of smokers [29]. For example, electrophilic metabolites of B[α]P generate DNA adducts with the nucleophilic groups of the two purine bases, adenine and guanine [30]. It has been reported that the genomic alterations observed *in vitro* after exposure of human breast epithelial cells to the tobacco carcinogen, B[α]P, resemble those seen in familial breast cancer [31]. Through comprehensive studies of the DNA adducts of B[α]P, a strong association between depurinating adducts and oncogenic mutations in the H-*ras* gene, which are generated by misrepair rather than misreplication of the apurinic sites, is suggested as the primary culprit in tumor initiation and progression [30]. Our results are consistent with these findings; however, we have not determined whether it is oncogenic mutation in H-*ras* responsible for neoplastic transformation of MCF10A cells after treatment with CSC or B[α]P.

The cigarette smoke also contains several DNA-alkylating agents that form *O* and *N* alkylation products of DNA bases. These *O* and *N* alkylation products of DNA bases are genotoxic in nature. Of these two types of DNA alkylation products, *N* alkylation lesions are predominant. The apurinic and *N* alkylation lesions are primarily removed from DNA by the BER pathway. There are two different BER pathways, i.e., short-patch (SP)–BER and long-patch (LP)–BER [32]. Germ-line knockouts, which have a deficiency in any one of the BER proteins, such as DNA polymerase β (Pol-β), AP endonuclease 1, 5′-flap endonuclease 1 (Fen1), or DNA ligase I, are all embryonically lethal, indicating the importance of BER in developmental process [33]. It has been estimated that approximately one million AP lesions are generated per mammalian cell per day [34]. AP lesions are unstable and degrade spontaneously into DNA strand breaks by β-elimination that retards DNA polymerases. The AP lesions are highly mutagenic because they result in nontemplate DNA and RNA synthesis. Despite the large number of AP lesions generated per cell per day, the number of mutations is extremely low because of BER, which is the main pathway for the repair of endogenous AP lesions. So, inhibition or deficiency of BER may result in enhanced accumulation of AP lesions that are mutagenic and carcinogenic in nature. Recently, we found that besides Pol-β, AP endonuclease 1, Fen1, and DNA ligase I, there is another protein APC that has a role in the regulation of BER [35–38].

Although *APC* is a known tumor suppressor gene, our findings suggest another role of APC that is related to DNA damage. Previously, we have shown that *APC* gene expression and protein levels are induced in cancer and normal breast and colonic epithelial cell lines upon exposure to the DNA-alkylating agents, *N*-methyl-*N*′-nitro-*N*-nitrosoguanine, methylmethane sulfonate and dimethylhydrazine, as well as the CSC and CSC carcinogens, dimethylbenzanthracene and B[α]P [25,39,40]. In the present study, we overexpressed and knocked down APC protein levels in MCF10A cells and treated them with CSC and B[α]P. We found a positive correlation of increased levels of APC with increased accumulation of AP lesions. Further, we found an enhanced neoplastic transformation of MCF10A cells in both *in vitro* and *in vivo* studies in which APC was overexpressed and challenged with CSC or B[α]P than APC-knockdown MCF10A cells. Our studies suggest that APC expression after DNA damage may lose its tumor suppressor function, instead block BER and stimulate carcinogenic process through accumulation of AP lesions.

In earlier studies, we have shown that the altered level of APC in different cell lines was associated with altered activity of BER [22]. We have described that APC interacts with Pol-β and blocks Pol-β–directed SP-BER by blocking its dRP-lyase activity [36]. We have further shown that APC also interacts with Fen1 and blocks LP-BER by blocking Pol-β–directed strand displacement synthesis, which is stimulated by Fen1 [27]. The blocked SP- and LP-BER can cause accumulation of AP lesions resulting in the transformation of target cells. In the present study, CSC and B[α]P treatments increased the level of APC with increased accumulation of AP lesions in mammary epithelial cells, which might be due to Pol-β and Fen1-mediated blockade of BER, as discussed in our previous studies [22,27,36,37]. These observations warrant further examination on population-based studies to firmly establish the role of APC in DNA repair and interaction with other mammary factors (such as hormones) with firsthand or secondhand cigarette smoke–induced breast carcinogenesis in susceptible and nonsusceptible populations. In addition, whether APC modulates DNA repair capacity of breast stem cells or affects only the differentiated cells in cigarette smoke–induced breast carcinogenesis is currently not known.

It is interesting to note that APC-knockdown MCF10A cells formed soft agar colonies after CSC and B[α]P treatments, although with a lesser extent than the MCF10A and APC-overexpressed MCF10A cells (Figure 4). The CSC and B[α]P-treated APC-knockdown MCF10A cells did not form tumors within 120 days compared to MCF10A cells when injected into the mammary pad of nude mice for the same period (Figure 5). This difference can be due to their acquired tumorigenic behavior and time required for the growth of tumors. It is possible that the 120-day time period for APC-knockdown MCF10A cells was not sufficient for tumorigenic growth. If these cells would have been left for additional 2 to 3 months, it is possible that we could have seen tumorigenic growth of these cells as well. Furthermore, the soft agar colony formation is not a true representation of the transformation characteristic of a cell. In previous studies, we established CSC-transformed MCF10A cell clones in culture. These cells made soft agar colonies, but one of four clones grew as tumor in nude mice. The clone that made tumor in nude mice had higher nonclonal chromosome aberrations than others, which serves as a marker for the probability of cancer progression [41]. Whether the difference in the growth of CSC- and B[α]P-treated MCF10A and APC-knockdown MCF10A cells in nude mice in the present study is due to differences in the numbers of nonclonal chromosome aberrations is not known. Since DNA damage–induced chromosomal aberrations are linked with carcinogenesis [42,43], it is likely that APC may play a role in CSC- and B[α]P-induced chromosomal aberrations in MCF10A cells by blocking BER and increasing AP lesions. In any case, it appears that APC knockdown inhibits the growth of mammary tumors after CSC and B[α]P treatments by maintaining the increased capacity of BER and thus the decreased level of AP lesions in these cells. In summary, we conclude that CSC and B[α]P treatments increase the APC level, which, in turn, blocks BER resulting in the accumulation of AP lesions in MCF10A cells. The accumulated AP lesions are

mutagenic and cause neoplastic transformation of these cells. Thus, these results provide experimental evidence that CSC, a surrogate for active or passive (secondhand) smoking, may cause neoplastic transformation of normal breast epithelial cells.

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