

# AP-2 $\alpha$ downregulation by cigarette smoke condensate is counteracted by p53 in human lung cancer cells

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**Abstract.** Cumulative findings have demonstrated that the dysregulation of tumor suppressor genes may be implicated in cigarette smoke-induced carcinogenesis. Activating enhancer-binding protein 2 (AP-2) is a eukaryotic transcriptional factor that plays a significant role in embryonic development and tumorigenesis. The vertebrate AP-2 family consists of AP-2 $\alpha$ , AP-2 $\beta$ , AP-2 $\gamma$ , AP-2 $\delta$  and AP-2 $\epsilon$ . Previous studies have suggested that cigarette smoking disrupts AP-2 regulation. In the present study, we investigated the effects of cigarette smoke condensate (CSC) on AP-2 $\alpha$  expression in human lung cancer cell lines (NCI-H1299, NCI-H446 and A549), as well as the potential mechanisms involved. Using RT-qPCR, we found that CSC decreased AP-2 $\alpha$  expression by suppressing its transcription in human lung cancer cell lines, particularly in p53-deficient NCI-H1299 cells. Western blotting and luciferase assays were implemented and we found that the restoration of p53 expression rescued the NCI-H1299 cells from CSC-induced AP-2 $\alpha$  loss, while the silencing of p53 resulted in increased AP-2 $\alpha$  loss induced by CSC, suggesting an antagonizing role of p53 in the regulation of AP-2 $\alpha$  by CSC. Our results indicate that AP-2 $\alpha$  downregulation may be involved in smoke-induced lung carcinogenesis.

## Introduction

Cigarette smoke has long been recognized as a risk factor associated with lung cancer. However, the underlying molecular mechanisms through which cigarette smoke induces lung carcinogenesis remain unclear.

Increasing evidence indicates that tumor suppressor genes play important roles in cigarette carcinogenesis. Nuclear receptor subfamily 1, group D member 1 (Nr1d1), also known as Rev-erb- $\alpha$ , a candidate tumor suppressor controlling cell proliferation, lipid metabolism and inflammation, has been found to be downregulated by cigarette smoke (1). Glypican 3, a glycosylphosphatidylinositol-linked heparan sulfate proteoglycan, functioning as a tumor suppressor by inhibiting cell growth and inducing apoptosis, is decreased in lung adenocarcinoma and in the normal lungs of smokers compared with the normal lungs of non-smokers (2). A number of the tumor suppressor genes are hypermethylated in the malignant lung tissues of smokers. Deleted in liver cancer 1 (DLC-1), homologous to RhoGAP, has been described as a novel tumor suppressor gene. A previous study demonstrated a significant association between DLC-1 methylation and cigarette smoke (3). The aberrant methylation of other tumor suppressor genes, such as p16 and fragile histidine triad (FHIT), is also found in smokers (4,5).

Activating enhancer-binding protein 2 (AP-2), a eukaryotic transcriptional factor, plays a pivotal role in normal development and morphogenesis during embryogenesis. The AP-2 family consists of 5 different isoforms, namely AP-2 $\alpha$ , AP-2 $\beta$ , AP-2 $\gamma$ , AP-2 $\delta$  and AP-2 $\epsilon$  (6). All AP-2 family members share a highly conserved helix-span-helix dimerization motif, a central basic region and a less conserved proline-rich domain. The AP-2 proteins can form homo- or heterodimers and transactivate target DNA. AP-2 $\alpha$  can regulate a number of tumor-related genes, such as p21 (7) human epidermal growth factor receptor 2 (HER-2) (8) and Bcl-2 (9). AP-2 $\alpha$  expression is reduced in many types of tumor tissue. The decreased expression of AP-2 $\alpha$  has been shown to significantly correlate with

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increased tumorigenic potential and poor prognosis (10,11), suggesting that AP-2 $\alpha$  is a candidate tumor suppressor.

Previous studies have identified AP-2 as an important transcriptional regulator of CYP11A1, a member of the cytochrome P450 family, which is an important metabolizing enzyme of cigarette smoke (12,13). Nicotine can suppress AP-2 $\alpha$  expression and its target DNA binding activity through the activation of peroxisome proliferator-activated receptor (PPAR), a key regulator of cancer cell proliferation (14). These findings prompted us to study the association between AP-2 $\alpha$  and cigarette smoke.

In the present study, we examined the role of AP-2 $\alpha$  in cigarette smoke and found that the presence of cigarette smoke condensate (CSC) suppressed AP-2 $\alpha$  expression in human lung cancer cell lines. AP-2 $\alpha$  promoter activity was markedly decreased in the presence of CSC. Furthermore, the overexpression or silencing of cellular p53 substantially affected the CSC-induced suppression of AP-2 $\alpha$ .

## Materials and methods

**Cell culture and transfection.** NCI-H1299, NCI-H446 and A549 human lung cancer cell lines were routinely cultured in complete medium containing RPMI-1640 + 10% fetal bovine serum (FBS). One day prior to transfection, the cells were seeded in 60-mm culture disks to reach 80-90% confluence at transfection. Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The transfection medium was substituted by complete medium 5 h after transfection.

**CSC preparation and treatment.** CSC was prepared as previously described (15). Briefly, cigarette smoke (from a popular type of cigarette, Honghe, 15 mg tar, 1.2 mg nicotine) was condensed in a 2-litre flask submerged in liquid nitrogen. The weight increase of the flask was representative of the amount of the smoke condensate. The condensate was dissolved in dimethyl sulfoxide (DMSO) up to 20 mg/ml, which was aliquoted and stored at -80°C. When used for the experiments, CSC was diluted in complete medium to the desired concentration. Control treatment was performed with complete medium containing an equivalent amount of DMSO.

**Plasmids and siRNA.** The promoter of AP-2 $\alpha$  spanning nucleotides -1728 to +286 was cloned from genomic DNA, as previously described (16). The p53-SN3 plasmid, which expresses wild-type p53 driven by a cytomegalovirus (CMV) promoter, was a kind gift from B. Vogelstein (Ohio State University, Columbus, OH, USA). AP-2-LUC, a luciferase reporter downstream of 3 repeats of AP-2 binding sites, was kindly provided by Carlo M. Croce (Ohio State University). p53-specific siRNA or scrambled siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

**RNA extraction and quantitative reverse transcription PCR (RT-qPCR).** Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Following quantification, 5  $\mu$ g of total RNA were reverse-transcribed into cDNA using the SuperScript™ First-Strand Synthesis System (Invitrogen). Quantitative PCR (qPCR) was performed using ABI PRISM 7000 (Applied Biosystems,

Foster City, CA, USA) and the SYBR® Premix Ex Taq™ II kit (Takara, Dalian, China). The detailed procedure followed the manufacturer's manual. The specific primer pairs used for qPCR were as follows: 5'-GATCCTCGCAGGGACTACAG-3' (sense) and 5'-TACCCGGGTCTTCTACATGC-3' for AP-2 $\alpha$ ; 5'-TGGTCACCAGGGCTGCTT-3' (sense) and 5'-AGCTTCCCGTTCTCAGCCTT-3' for GAPDH. GAPDH was used as an internal control. The AP-2 $\alpha$  relative expression levels were calculated using the  $\Delta\Delta$ CT method. Changes in AP-2 $\alpha$  expression levels were expressed as a percentage change compared to the corresponding control (defined as 100%). All experiments were performed in triplicate and repeated thrice.

**Protein preparation and western blot analysis.** Total proteins were extracted using modified RIPA buffer and quantified using Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Total proteins (40  $\mu$ g) were loaded and run on a 10% SDS-PAGE gel. Following electrophoresis, the proteins were semi-dry transferred onto nitrocellulose membranes. After blocking in 5% non-fat milk and washing with PBS with 0.1% Tween-20, the membranes were incubated with the appropriately diluted primary antibodies against AP-2 $\alpha$  or  $\beta$ -actin (all from Santa Cruz Biotechnology) at 4°C overnight. Following incubation with HRP-conjugated secondary antibody, the HRP signal was detected using Super ECL Plus Detection reagent (Appligen Technologies Inc., Beijing, China) and exposed to X-ray film.

**Luciferase reporter assay.** The Dual-Luciferase® Reporter Assay System (Promega, Madison, WI, USA) was used to detect AP-2 $\alpha$  promoter activity. Briefly, the cells were co-transfected with the test plasmids or the empty pGL3-Basic vector and pRL-SV40 as controls (Promega). Following transfection and CSC treatment, the cells were washed in PBS and harvested using passive lysis buffer. The enzyme activities of firefly luciferase and *Renilla* luciferase were measured sequentially using the Dual-Luciferase® Reporter Assay System (Promega). The ratio of firefly luciferase levels to *Renilla* luciferase levels was used as the relative luciferase activity. Changes in luciferase activity were expressed as percentage change compared to the corresponding control (defined as 100%). All experiments were performed in triplicate and repeated thrice.

**Statistical analysis.** All qPCR results are presented as the means  $\pm$  standard error. The Student's t test was used to identify significant differences between groups. Statistical significance was set at  $P < 0.05$ .

## Results

**CSC inhibits AP-2 $\alpha$  expression in lung cancer cell lines.** We first investigated the effects of CSC on AP-2 $\alpha$  mRNA expression. Three human lung cancer cell lines NCI-H1299, NCI-H446 and A549, were cultured to 70% confluence and then incubated with 10  $\mu$ g/ml CSC or DMSO for different periods of time. RT-qPCR was used to detect the mRNA levels of AP-2 $\alpha$ . As shown in Fig. 1A, CSC significantly decreased AP-2 $\alpha$  mRNA expression in the NCI-H1299 cells in a time-dependent manner. AP-2 $\alpha$  mRNA expression slightly decreased after 8 h of exposure to CSC and then remained decreased until it reached its lowest level at 24 h, at which time the AP-2 $\alpha$  level

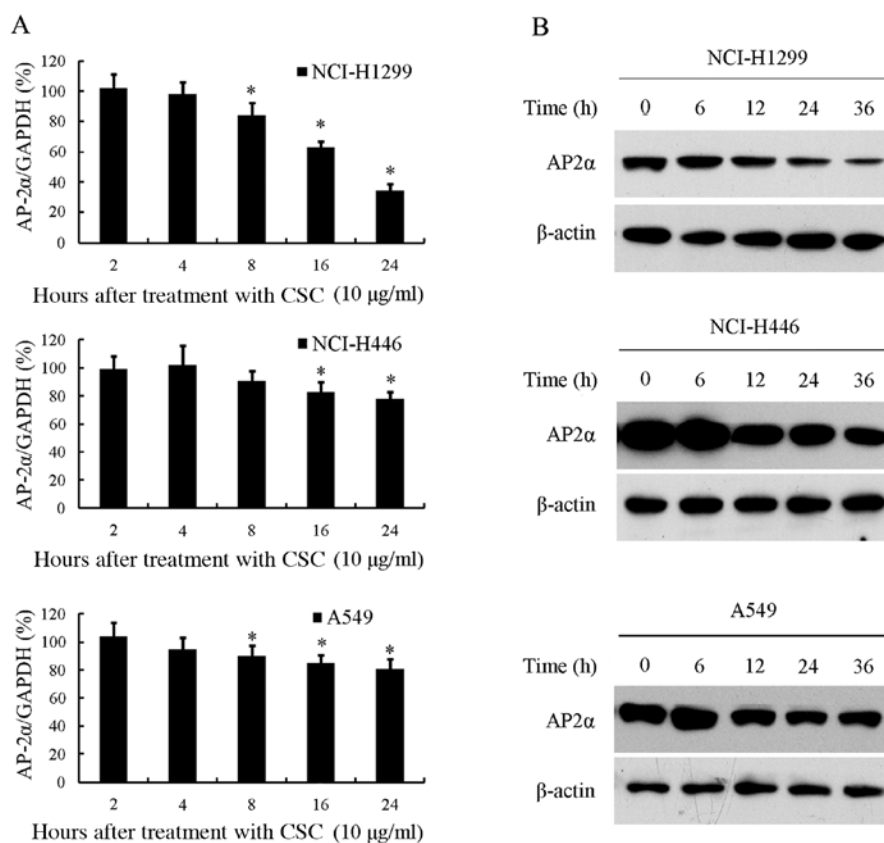


Figure 1. Activating enhancer-binding protein 2α (AP-2α) expression following treatment with cigarette smoke condensate (CSC) in lung cancer cell lines. The human lung cancer cell lines, NCI-H1299, NCI-H446 and A549, were cultured to 70% confluence and then incubated with 10 µg/ml CSC or DMSO for several hours, as indicated. Following incubation, (A) RT-qPCR and (B) western blot analysis were used to detect the AP-2α mRNA and protein expression. \*P<0.05, compared to the untreated cells.

was decreased to 34% compared to the untreated controls (P<0.05). AP-2α mRNA expression was also inhibited by exposure to CSC in the NCI-H446 and A549 cells (P<0.05), although to a much lesser extent. DMSO treatment did not alter the AP-2α mRNA expression levels (data not shown).

Subsequently, we examined AP-2α protein expression following incubation with CSC. In parallel to the mRNA results, we also found that AP-2α protein expression was markedly inhibited by CSC, but not by DMSO (data not shown), in the NCI-H1299 cells, and to a much lesser extent in the NCI-H446 and A549 cells (Fig. 1B).

*CSC inhibits the transactivating ability of AP-2α, but does not affect its subcellular localization.* To evaluate the functional changes of AP-2α which occur due to smoke, we examined the transactivating ability of AP-2α following exposure to CSC. The NCI-H1299, NCI-H446 and A549 cells were transfected with the empty vector, pGL3-basic, or AP-2-LUC, a luciferase reporter downstream of 3 repeats of AP-2 binding sites. At 24 h after transfection, the cells were incubated with 10 µg/ml CSC or DMSO. After a further 24 h of incubation, luciferase activity was detected. As shown in Fig. 2A, the luciferase activity of AP-2-LUC in the NCI-H1299 cells was reduced by CSC, but not by DMSO, while no significant changes in pGL3-basic luciferase activity were observed in the presence of CSC or DMSO. Similar results were also observed in the NCI-H446 and A549 cells, but not as profoundly as in the NCI-H1299

cells. The results coincided with the observations that CSC downregulated the expression of AP-2α.

To exclude the possibility that the decreased transactivating ability of AP-2α induced by CSC may result from the changes in its subcellular localization, we examined the subcellular localization of AP-2α following exposure of the cells to CSC by immunofluorescence. As shown in Fig. 2B, AP-2α was mainly localized in the nucleus under normal growth conditions. Treatment with 24 h with 10 µg/ml CSC did not alter its subcellular localization; however, its expression levels were decreased, which is in agreement with the above results.

*CSC suppresses the promoter activity of AP-2α.* To determine whether the CSC-induced downregulation of AP-2α occurs at its transcriptional level, we constructed a luciferase reporter plasmid, pGL3-AP-2α, containing a putative AP-2α promoter spanning from position -1728 to +286. The NCI-H1299 cells were transfected with pGL3-AP-2α or the control plasmid, pGL3-basic, and 24 h later the cells were treated with 10 µg/ml CSC or DMSO for 24 h. Unsurprisingly, CSC treatment significantly reduced the ability of the AP-2α promoter to drive the luciferase expression in the NCI-H1299 cells (P<0.05) (Fig. 3A). However, the inhibitory effect was not pronounced, but statistically significant, in the NCI-H446 and A549 cells (Fig. 3B and C).

*Transfection of p53 inhibits the CSC-induced downregulation of AP-2α by rescuing its promoter activity in NCI-H1299*

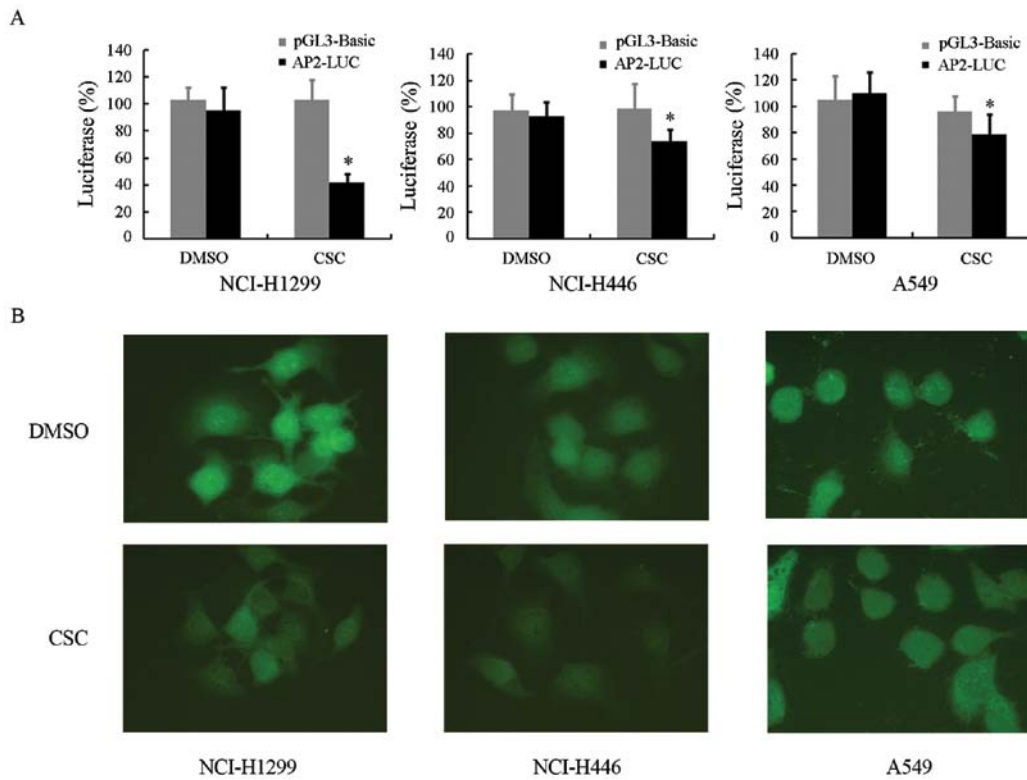


Figure 2. Activating enhancer-binding protein 2 $\alpha$  (AP-2 $\alpha$ ) transactivating ability and subcellular localization following treatment with cigarette smoke condensate (CSC) in lung cancer cell lines. (A) The human lung cancer cell lines, NCI-H1299, NCI-H446 and A549, were cultured to 90% confluence and co-transfected with pRL-SV40 and pGL3-basic or AP-2-LUC for 24 h. The cells were then incubated with 10  $\mu$ g/ml CSC or DMSO for 24 h and the luciferase activity of both in the cell lysates was determined. The activity of firefly luciferase was normalized to *Renilla* luciferase and is expressed as a percentage change compared to the untreated controls (mean  $\pm$  SD of 3 independent experiments). \*P<0.05, compared to the untreated cells. (B) Cells were treated as indicated in Fig. 1 and immunofluorescence was performed to analyze the subcellular localization of AP-2 $\alpha$ .

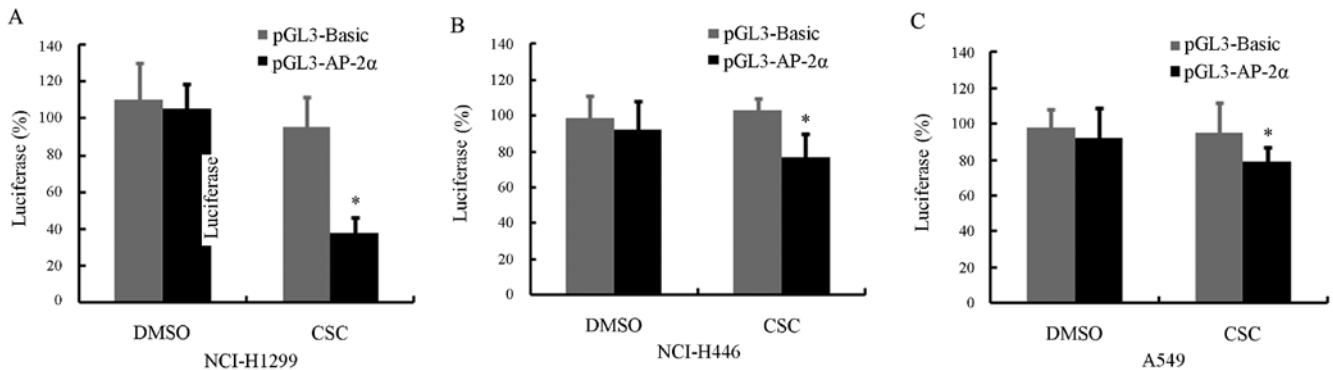


Figure 3. Promoter activity of activating enhancer-binding protein 2 $\alpha$  (AP-2 $\alpha$ ) following treatment with cigarette smoke condensate (CSC) in lung cancer cell lines. The NCI-H1299, NCI-H446 and A549 cells were treated as indicated in Fig. 2A, except that pGL3-AP-2 $\alpha$  was used instead of AP-2-LUC. \*P<0.05, compared to the untreated cells.

cells. The above-mentioned results showed a differential effect of CSC on different human lung cancer cell lines. The NCI-H1299 cell line was more sensitive to the CSC-induced inhibition of AP-2 $\alpha$  expression. As is already known, NCI-H1299 is a p53-deficient cell line, whereas NCI-H446 and A549 cells harbor wild-type p53. This prompted us to examine whether p53 plays a role in the CSC-mediated downregulation of AP-2 $\alpha$ . Therefore, we restored p53 expression in the NCI-H1299 cells and investigated its effects on the CSC-induced downregulation of AP-2 $\alpha$ . The wild-type p53 plasmid, p53-SN3, or the empty vector, pCMV, was transiently

transfected into the NCI-H1299 cells. After 24 h, the cells were incubated with 10  $\mu$ g/ml CSC or DMSO for 24 h. As shown in Fig. 4A, in the mock-transfected cells (empty vector), we did not detect any p53 expression, while p53 expression was clearly detected following transfection with p53-SN3. As expected, p53 transfection attenuated the loss of AP-2 $\alpha$  induced by exposure to CSC. We further analyzed AP-2 $\alpha$  mRNA levels. As observed in the above-mentioned experiments, AP-2 $\alpha$  mRNA expression was markedly inhibited by exposure to CSC in both the mock-transfected and p53-SN3-transfected cells (P<0.05). However, the p53-transfected cells showed a much higher

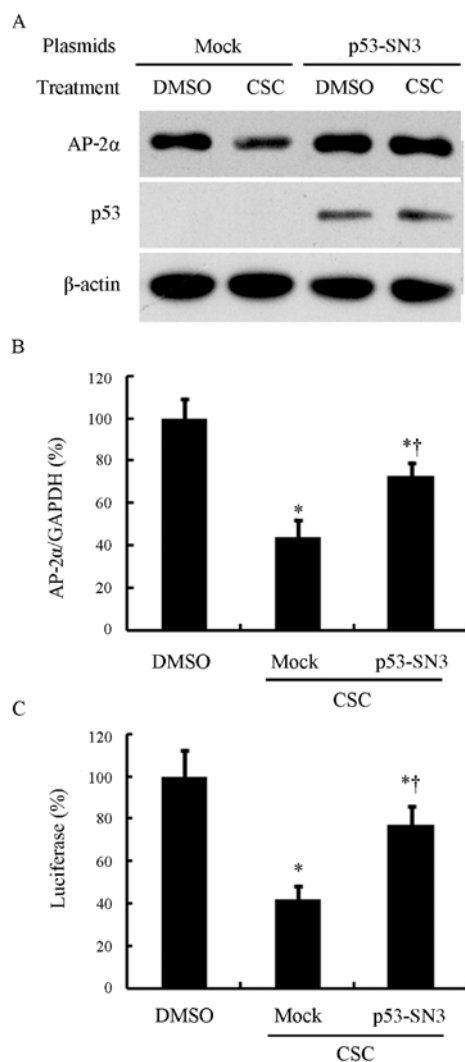


Figure 4. Activating enhancer-binding protein 2α (AP-2α) expression and its promoter activity following treatment with CSC in the p53-transfected NCI-H1299 cells. The NCI-H1299 cells were transfected with p53-SN3 or the empty vector, pCMV-Neo-Bam. Twenty-four hours after transfection, the cells were treated with 10 μg/ml cigarette smoke condensate (CSC) or DMSO for 24 h. (A) Western blot analysis was used to detect the p53 and AP-2α expression status. (B) qPCR was used to detect the AP-2α mRNA expression. \*P<0.05, compared to the DMSO-treated cells. †P<0.05, compared to the mock-transfected cells. (C) The NCI-H1299 cells were co-transfected with pRL-SV40 and pGL3-AP-2α and p53-SN3 or pCMV-Neo-Bam. Twenty-four hours after transfection, the cells were treated with 10 μg/ml CSC or DMSO for 24 h and the luciferase activity of both was determined. \*P<0.05, compared to the DMSO-treated cells. †P<0.05, compared to the mock-transfected cells.

AP-2α expression compared with the mock-transfected cells (P<0.05) (Fig. 4B).

Subsequently, we investigated the effects of p53 transfection on AP-2α promoter activity following exposure to CSC. pGL3-AP-2α and the control plasmid, pRL-SV40, were co-transfected into the NCI-H1299 cells with p53-SN3 or the empty vector, pCMV. After 24 h, the cells were incubated with 10 μg/ml CSC or DMSO for 24 h and the luciferase activity was determined. As expected, p53 partially reversed the suppression of the AP-2α promoter induced by CSC, while the mock-transfected cells still showed a significant loss of AP-2α following treatment with CSC (Fig. 4C).

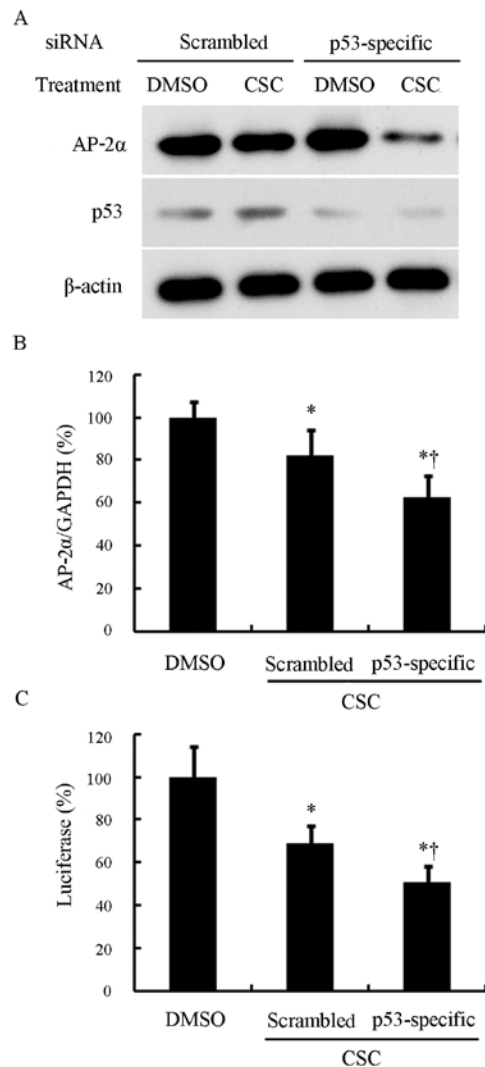


Figure 5. Activating enhancer-binding protein 2α (AP-2α) expression and its promoter activity following treatment with cigarette smoke condensate (CSC) in p53-silenced A549 cells. The A549 cells were transfected with p53-specific siRNA or scrambled siRNA. Twenty-four hours after transfection, the cells were treated with 10 μg/ml CSC or DMSO for 24 h. (A) Western blot analysis was used to detect the p53 and AP-2α expression status. (B) qPCR was used to detect AP-2α mRNA expression. \*P<0.05, compared to the DMSO-treated cells. †P<0.05, compared to the mock-transfected cells. (C) The NCI-H1299 cells were co-transfected with pRL-SV40 and pGL3-AP-2α and p53-specific siRNA or scrambled siRNA. Twenty-four hours after transfection, the cells were treated with 10 μg/ml CSC or DMSO for 24 h and the luciferase activity of both was determined. \*P<0.05, compared to the DMSO-treated cells. †P<0.05, compared to the mock-transfected cells.

*p53 silencing enhances the CSC-induced downregulation of AP-2α in A549 cells.* To further confirm the involvement of p53 in the downregulation of AP-2α by CSC, we silenced p53 in the A549 lung cancer cells using p53-specific siRNA (Fig. 5A). As expected, following treatment with CSC, the p53-specific siRNA-transfected cells and scrambled siRNA-transfected cells both showed a decrease in the AP-2α mRNA expression level (P<0.05) (Fig. 5B). However, the p53-specific siRNA-transfected cells expressed much lower levels of AP-2α than the scrambled siRNA-transfected cells following treatment with CSC (P<0.05) (Fig. 5B). In addition, the silencing of p53 intensified the inhibition of the AP-2α

promoter induced by CSC (Fig. 5C). These results suggest a counteracting role of p53 in the CSC-induced AP-2 $\alpha$  downregulation.

## Discussion

Cigarette smoke is a complex mixture of which more than 4,800 compounds have been identified (17), over 55 of which have been implicated in lung cancer, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[a]pyrene (B[a]P) and acrolein (18,19). Polycyclic aromatic hydrocarbons (PAHs), one of the most studied tobacco toxins, is present in the combustion products of cigarette smoke and have long been considered to be an important carcinogen responsible for the initiation and development of lung cancer (20). PAHs can be activated by cytochrome P450 enzymes to form its metabolite, diol epoxides, which induce DNA mutation by forming covalent adducts with DNA (21).

Studies using DNA microarray have revealed that cigarette smoke can induce significant changes in the transcriptome. Spira *et al* (22), using high-density gene expression arrays, found that the normal airway transcriptome consists of more than 7,000 measurable genes, while smoking alters the expression of numerous genes. Although the levels of most of the susceptible genes return to normal after smoking cessation, several potential oncogenes (such as CEACAM6 and HN1) are permanently increased and tumor suppressor genes (such as TU3A and CX3CL1) are permanently decreased. Another study, using serial analysis of gene expression (SAGE) also verified many reversible and irreversible gene expression changes upon the cessation of smoking and the irreversible changes may lead to persistent lung cancer risk despite smoking cessation (23). In the present study, we demonstrated that AP-2 $\alpha$ , one of the putative tumor suppressors, was downregulated by CSC in lung cancer cell lines, suggesting the involvement of the loss of AP-2 $\alpha$  in smoking-related lung cancer development.

It has long been suspected that the loss of AP-2 may be associated with tumorigenesis. Early investigations on primary invasive breast cancers demonstrated that AP-2 expression is reduced in the majority of tumors, suggesting that AP-2 acts as a tumor suppressor during breast carcinogenesis (24). Similar findings have also been reported for prostate and colon cancers. The enforced AP-2 overexpression in melanoma and prostate cancer cell lines has been shown to lead to reduced tumorigenicity (25,26). Functional studies have indicated that AP-2 $\alpha$  can bind as a homo- or heterodimer to the motif 'GCCNNNGGC' in the regulatory regions of its target genes. After binding, AP-2 $\alpha$  can activate the transcription of target genes (27). In melanoma, AP-2 $\alpha$  may function as a tumor suppressor by directly activating the transcription of downstream genes, such as c-KIT, E-cadherin, protease activated receptor 1 (PAR-1), matrix metalloproteinase-2 (MMP-2) and vascular endothelial growth factor (VEGF) (28). AP-2 $\alpha$  can also act as transcriptional repressor. A previous study demonstrated that AP-2 $\alpha$  competes with SP1 for binding to the CYP17 gene promoter to repress its transcription (29). Epigenetic studies have revealed that AP-2 $\alpha$  can recruit histone deacetylase (HDAC) to the promoter regions of target genes, thus silencing these genes (30). Another study demonstrated that frequent meth-

ylation regions in acute lymphoblastic leukemia contain multiple AP-2 $\alpha$  binding sites, suggesting a potential role of AP-2 $\alpha$  in epigenetic regulation (31).

To determine at which level AP-2 $\alpha$  downregulation occurs following exposure to CSC, we performed luciferase reporter assays and revealed a reduced promoter activity of AP-2 $\alpha$  by CSC treatment. These findings suggest that the CSC-induced downregulation of AP-2 $\alpha$  occurs at the transcriptional level. To date, the mechanisms underlying the transcriptional regulation of AP-2 expression remain obscure. A previous study investigated the transcriptional regulation of the AP-2 $\alpha$  gene promoter and found that many nuclear proteins can bind to the AP-2 $\alpha$  promoter (32). The authors also found that BTEB-1 and AP-2rep are two transcriptional regulators of AP-2 $\alpha$ . BTEB-1 acts as a positive regulator, while the function of AP-2rep depends on its binding *cis*-elements. Another study identified an enhancer in the AP-2 $\alpha$  promoter, which contains an Ets-1 binding site (16). Strikingly, AP-2 $\alpha$  is subjected to autoregulation since an AP-2 $\alpha$  binding site exists in the AP-2 $\alpha$  promoter region (33). AP-2 expression is also regulated at the post-transcriptional level. A previous study demonstrated that the AP-2 $\alpha$  protein is unusually stable in HER-2-positive breast cancer cell lines, which results from the insufficient proteasomal-degradation of AP-2 $\alpha$  (34).

As the changes in AP-2 $\alpha$  levels were more pronounced in p53-deficient NCI-H1299 cells than in NCI-H446 and A549 cells which harbor wild-type p53, we transfected a wild-type p53 expression plasmid into the NCI-H1299 cells to examine the changes in the levels of AP-2 $\alpha$  following exposure of the cells to CSC. The results revealed that the p53 restoration antagonized the effects of the CSC-induced decrease in AP-2 $\alpha$  expression. In addition, we observed an enhanced ability of CSC to decrease AP-2 $\alpha$  expression in the A549 cells silenced by p53 siRNA. All these findings suggest that p53 antagonizes CSC-induced AP-2 $\alpha$  downregulation. It has previously been demonstrated that AP-2 $\alpha$  and AP-2 $\gamma$  are transcriptionally regulated by p53 (35). p53 activates AP-2 $\alpha$  and AP-2 $\alpha$  transcription by binding to and remodeling their promoters (35). Although we did not observe an obvious increase in the AP-2 $\alpha$  level following p53 transfection, p53 restoration can counteract the CSC-induced AP-2 $\alpha$  downregulation. Other studies have reported that AP-2 $\alpha$  can also regulate the transcriptional activity of p53 through co-activation and decreased stability (36). The overexpression of AP-2 $\alpha$  can repress p53-mediated p21 activation (36). All these findings suggest a reciprocal regulation between p53 and AP-2 $\alpha$ .

Taken together, the results of the present study provide new mechanistic information as to the mechanisms through which cigarette smoke exerts cytotoxic and tumorigenic effects on lung cells. However, given the complexity of cigarette smoke, it is difficult to identify the specific AP-2 $\alpha$ -killing culprits. Further studies are required in order to screen potential cigarette toxins.

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