ARTICLE
Journal of Cellular Biochemistry 112:589–599 (2011)

Journal of Cellular Biochemistry

Cigarette Smoke Extract Regulates Cytosolic Phospholipase A_2 Expression Via NADPH Oxidase/MAPKs/AP-1 and p300 in Human Tracheal Smooth Muscle Cells

Shin-Ei Cheng,[1] Chih-Chung Lin,[2] I-Ta Lee,[1] Chih-Kai Hsu,[1] Yu Ru Kou,[3] and Chuen-Mao Yang[1*]

[1]*Department of Physiology and Pharmacology, Chang Gung University, Tao-Yuan, Taiwan*
[2]*Department of Anesthetics, Chang Gung Memorial Hospital and Chang Gung University, Tao-Yuan, Taiwan*
[3]*Department of Physiology, National Yang Ming University, Taipei, Taiwan*

ABSTRACT

Up-regulation of cytosolic phospholipase A_2 ($cPLA_2$) by cigarette smoke extract (CSE) may play a critical role in airway inflammatory diseases. However, the mechanisms underlying CSE-induced $cPLA_2$ expression in human tracheal smooth muscle cells (HTSMCs) were not completely understood. Here, we demonstrated that CSE-induced $cPLA_2$ protein and mRNA expression was inhibited by pretreatment with the inhibitors of AP-1 (tanshinone IIA) and p300 (garcinol) or transfection with siRNAs of c-Jun, c-Fos, and p300. Moreover, CSE also induced c-Jun and c-Fos expression, which were inhibited by pretreatment with the inhibitors of NADPH oxidase (diphenyleneiodonium chloride and apocynin) and the ROS scavenger (N-acetyl-L-cysteine) or transfection with siRNAs of $p47^{phox}$ and NADPH oxidase (NOX)2. CSE-induced c-Fos expression was inhibited by pretreatment with the inhibitors of MEK1 (U0126) and p38 MAPK (SB202190) or transfection with siRNAs of p42 and p38. CSE-induced c-Jun expression and phosphorylation were inhibited by pretreatment with the inhibitor of JNK1/2 (SP600125) or transfection with JNK2 siRNA. CSE-stimulated p300 phosphorylation was inhibited by pretreatment with the inhibitors of NADPH oxidase and JNK1/2. Furthermore, CSE-induced p300 and c-Jun complex formation was inhibited by pretreatment with diphenyleneiodonium chloride, apocynin, N-acetyl-L-cysteine or SP600125. These results demonstrated that CSE-induced $cPLA_2$ expression was mediated through NOX2-dependent p42/p44 MAPK and p38 MAPK/c-Fos and JNK1/2/c-Jun/p300 pathways in HTSMCs. J. Cell. Biochem. 112: 589–599, 2011.

KEY WORDS: CIGARETTE SMOKE EXTRACT; TRACHEAL SMOOTH MUSCLE CELLS; REACTIVE OXYGEN SPECIES; SIGNALING TRANSDUCTION; CYTOSOLIC PHOSPHOLIPASE A2

Cigarette smoking is a risk factor in the pathogenesis of chronic obstructive pulmonary disease (COPD) which is characterized by inflammatory responses in the lungs [Moodie et al., 2004]. Moreover, the detrimental effects of cigarette smoke (CS) in lung inflammation may result from enhancing prostaglandin (PG) generation in the lungs [Martey et al., 2005]. Airway smooth muscle (ASM) is considered as an end-response effector mediating regional differences in ventilation by contraction in response to various pro-inflammatory mediators and exogenous substances under homeostatic or pathologic conditions [Hirst et al., 2004].

A number of lipid mediators, such as ecosanoids generated from arachidonic acid (AA) have been identified in situ in airway secretion of asthmatics [Barnes, 1989]. These AA metabolites play an important role in the inflammatory pathogenesis, including asthma [Henderson et al., 2002]. The generation of ecosanoids is first initiated through the release of AA from membrane phospholipids hydrolyzed by the action of phospholipase A_2 (PLA_2) enzymes [Balsinde et al., 1999; Bonventre and Sapirstein, 2002]. AA is further converted to PGs, such as PGE_2 by the constitutive enzyme cyclooxygenase (COX)-1 or the inducible COX-2 in various cell types [DeWitt, 1999; Yang et al., 2002]. The PLA_2 superfamily is composed of three main types of lipolytic enzymes, including secretory PLA_2, the 85 kDa cytosolic group IV PLA_2 ($cPLA_2$), and a calcium-independent group VI PLA_2 in mammalian cells [Six and Dennis, 2000]. Although several subtypes of PLA_2 have been

Grant sponsor: National Science Council, Taiwan; Grant numbers: NSC98-2314-B-182-021-MY3, NSC96-2320-B-182-003, NSC96-2320-B-182-047-MY3; Grant sponsor: Chang Gung Medical Research Foundation, Taiwan; Grant numbers: CMRPD32043, CMRPD170332, CMRPG381522, CMRPG350653, CMRPG360513.

*Correspondence to: Chuen-Mao Yang, PhD, Department of Pharmacology, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-San, Tao-Yuan, Taiwan. E-mail: chuenmao@mail.cgu.edu.tw

Received 27 October 2010; Accepted 28 October 2010 • DOI 10.1002/jcb.22949 • © 2010 Wiley-Liss, Inc.
Published online 22 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

described, $cPLA_2$ is the only one that exhibits specificity for AA, and its role in mediating agonist-induced AA release for eicosanoid production in various cell types is well studied [Leslie, 1997]. $cPLA_2$ has been shown to be implicated in acute lung injury induced by sepsis [Nagase et al., 2000] and bronchial reactivity associated with anaphylaxis [Uozumi et al., 1997]. Furthermore, increased synthesis of PGE_2 is dependent on an increase in $cPLA_2$ activity in various cell types [Dieter et al., 2002; Ghosh et al., 2004]. Tracheal smooth muscle cells also synthesize these lipid mediators that exert physiological or pathological actions under various conditions. These results demonstrate that $cPLA_2$ plays an important role in mediating AA release for production of PGE_2 by inflammatory cells and airway resident cells.

Furthermore, CS has been implicated in initiating inflammatory responses in the airways through the activation of transcription factors, such as NF-κB, AP-1, and other signaling transduction pathways, such as MAPKs, leading to enhancing expression of inflammatory genes [Rahman and Adcock, 2006]. In a previous study, we have shown that cigarette smoke extract (CSE) enhances the generation of ROS and induces the expression of $cPLA_2$ in human tracheal smooth muscle cells (HTSMCs) through the activation of NADPH oxidase/MAPKs/NF-κB and AP-1 mechanisms [Cheng et al., 2009]. AP-1 is a transcriptional regulator and composed of members of the Fos and Jun families of DNA binding proteins [Zenz et al., 2008]. The Fos proteins (Fos, FosB, Fra-1, and Fra-2) can only heterodimerize with members of the Jun family. In contrast, the Jun family members can homodimerize themselves and heterodimerize with Fos members. Moreover, MAPKs have been shown to regulate AP-1 activation [Zenz et al., 2008]. Oxidative stress is an important initiator in the pathogenesis of COPD. We have also shown that CSE-induced MAPKs activation is mediated through NADPH oxidase/ROS in HTSMCs [Cheng et al., 2009]. Moreover, ROS have been shown to regulate AP-1 activation [Cheng et al., 2009]. These findings imply that these signaling components such as AP-1 subunits (c-Fos and c-Jun) might be independently or cooperatively implicated in the expression of $cPLA_2$ induced by CSE in HTSMCs.

Histone acetyltransferases (HATs), such as p300 and CBP (CREB-binding protein) functioning as transcriptional co-activators and signal integrators have been proven to play a vital role in expression of inflammatory genes. Our previous study has demonstrated that IL-1β induced p300 activation and recruitment to the promoter region of $cPLA_2$ [Luo et al., 2008]. In addition, CS has been shown to increase histone acetylation in A549 cells [Moodie et al., 2004]. Although several lines of evidence address the involvement of AP-1 and p300 in $cPLA_2$ expression, the roles of p300 and AP-1 (c-Fos and c-Jun) in $cPLA_2$ expression in response to CSE remained to be determined.

Therefore, the experiments were performed to investigate the mechanisms of intracellular signaling pathways involved in CSE-induced $cPLA_2$ expression in HTSMCs. We report here that in HTSMCs, CSE-induced $cPLA_2$ expression was, at least in part, mediated through NADPH oxidase/MAPKs/c-Foc and c-Jun as well as p300.

MATERIALS AND METHODS

MATERIALS

PP1, diphenylene iodonium chloride (DPI), U0126, SB202190, SP600125, and tanshinone IIA were from Biomol (Plymouth Meeting, PA). Anti-$cPLA_2$, anti-c-Jun, anti-c-Fos, anti-p300, anti-p42, anti-p38, anti-JNK2, anti-phospho-p300, anti-$p47^{phox}$, and anti-NOX2 antibodies were from Santa Cruz (Santa Cruz, CA). Anti-phospho-c-Jun antibody was from Cell Signaling (Danver, MA). N-acetyl-L-cysteine (NAC) was from Sigma (St. Louis, MO). Apocynin (APO) was purchased from ChromaDex (Santa Ana, CA). Luciferase assay kit was from Promega (Madison, WI).

PREPARATION OF CIGARETTE SMOKE EXTRACT

CSE was prepared by a method previously described with some modifications [Mur et al., 2004; Ramage et al., 2006; Nagaraj and Zacharias, 2007]. In brief, 10 cigarettes (Long Life™, Taiwan Tobacco and Liquor Production, Taipei, Taiwan; 0.9 mg nicotine/cigarette) were combusted and the smoke was sucked through a standard glass-fiber Cambridge filter with a constant flow (0.3 L/min) by an air compressor. The Cambridge filters have been reported to trap >99% of the smoke particulate matter [Mur et al., 2004]. The smoke filter was quickly dried with a hot plate and the raised weight in each filter was defined as the amount of CSE. On average, each CS generated ~39.5 mg of CSE using this method. The CSE was dissolved by soaking the filter in DMSO for 30 min at room temperature. The solution containing CSE was centrifuged and the supernatant was collected and filtered using a 0.22-μm filter column (Millipore, Bedford, MA). The CSE stock solution (20 mg/ml of CSE containing 0.36 mg/ml of nicotine) was kept in microtubes (each 15 μl) and was immediately stored in a −80°C freezer. Before each experiment, the frozen CSE stock solution was defrosted and further diluted to the desired concentration with cell medium. The quality of the CSE solution was assessed based on the absorbance at 302 nm, which is the specific absorption spectrum of peroxynitrite. Our preliminary study indicated that DMSO less than 1% had no effect on HTSMCs. Therefore, the concentration of DMSO in the testing solution was always <1% to prevent possible cytotoxicity or other effects.

CELL CULTURE

HTSMCs were purchased from ScienCell Research Lab (San Diego, CA) and grown as previously described [Lee et al., 2008]. Experiments were performed with cells from passages 4–8. The cytotoxicity of each inhibitor at the incubation time was checked using an XXT assay kit. The cell viability was more than 90% after treatment with DMSO, these inhibitors, and/or CSE during the period of observation (data not shown).

TRANSIENT TRANSFECTION WITH SIRNAS

SMARTpool RNA duplexes corresponding to human NOX2, $p47^{phox}$, p38, p42, JNK2, c-Jun, c-Fos, p300, and scrambled #2 siRNA were from Dharmacon Research, Inc. (Lafayette, CO). Transient transfection of siRNAs was carried out using Metafectene transfection reagent. siRNA (100 nM) was formulated with Metafectene transfection reagent according to the manufacturer's instruction.

WESTERN BLOT ANALYSIS

Growth-arrested HTSMCs were incubated with CSE at 37°C for the indicated times. The cells were washed, scraped, collected, and centrifuged at 45,000*g* at 4°C for 1 h to yield the whole cell extract,

as previously described [Lee et al., 2008]. Samples were denatured, subjected to SDS–PAGE using a 12% running gel, transferred to nitrocellulose membrane, incubated with an anti-$cPLA_2$ antibody for 24 h, and then incubated with an anti-mouse horseradish peroxidase antibody for 1 h. The immunoreactive bands detected by ECL reagents were developed by Hyperfilm-ECL.

RT-PCR ANALYSIS

Total RNA was isolated with Trizol according to the protocol of the manufacturer. The cDNA obtained from 0.5 μg total RNA was used as a template for PCR amplification as previously described [Lee et al., 2008]. The primers used were as follows:

β-Actin
5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′ (sense)
5′-CTAGAAGCATTTGCGGTGGACGATG-3′ (anti-sense)
$cPLA_2$
5′-CTCACACCACAGAAAGTTAAAAGAT-3′ (sense)
5′-GCTACCACAGGCACATCACG-3′ (anti-sense)
c-Jun
5′-GGTACAAGGCGGAGAGGAAG-3′ (sense)
5′-GCGTTAGCATGAGTTGGCAC-3′ (anti-sense)
c-Fos
5′-GGAGAATCCGAAGGGAAAGG-3′ (sense)
5′-GCTTGGGCTCAGGGTCATTG-3′ (anti-sense)

ISOLATION OF CELL FRACTIONS

Cells were harvested, sonicated for 5 s at output 1.5 with a sonicator (Misonix, Farmingdale, NY), and centrifuged at 8,000 rpm for 15 min at 4°C. The pellet was collected as the nuclear fraction. The supernatant was centrifuged at 14,000 rpm for 60 min at 4°C to yield the pellet (membrane fraction) and the supernatant (cytosolic fraction).

CO-IMMUNOPRECIPITATION ASSAY

Cell lysates containing 1 mg of protein were incubated with 2 μg of anti-p300 or anti-c-Jun antibody at 4°C for 24 h, and then 10 μl of 50% protein A-agarose beads was added and mixed at 4°C for 24 h. The immunoprecipitates were collected and washed three times with a lysis buffer without Triton X-100. Five times Laemmli buffer was added, subjected to electrophoresis on 12% SDS–PAGE, transferred to nitrocellulose membrane, and then blotted using an anti-c-Jun or anti-p300 antibody.

CHROMATIN IMMUNOPRECIPITATION ASSAY

To detect the association of nuclear proteins with human $cPLA_2$ promoter, chromatin immunoprecipitation (ChIP) analysis was conducted as previously described [Nie et al., 2003]. DNA immunoprecipitated by using an anti-p300 or anti-c-Jun was purified. The DNA pellet was re-suspended in H_2O and subjected to PCR amplification. PCR products were analyzed on ethidium bromide-stained agarose gels.

MEASUREMENT OF $cPLA_2$ LUCIFERASE ACTIVITY

For construction of the $cPLA_2$-luc plasmid, human $cPLA_2$ promoter, a region spanning −2,375 to +75 bp, was cloned into pGL3-basic vector (Promega). $cPLA_2$-luc activity was determined as previously described [Lee et al., 2008] using a luciferase assay system (Promega). Firefly luciferase activities were standardized for β-gal activity.

ANALYSIS OF DATA

All the data were estimated using the GraphPad Prism Program (GraphPad, San Diego, CA). Data were expressed as the mean ± SEM and analyzed by one-way ANOVA followed with Tukey's post hoc test. $P < 0.05$ was considered significant.

RESULTS

CSE INDUCES $cPLA_2$ EXPRESSION VIA AP-1

The importance of AP-1 on the regulation of $cPLA_2$ gene expression is strongly supported by finding the AP-1 binding motif on $cPLA_2$ promoter region [Morri et al., 1994]. Thus, the role of AP-1 in CSE-induced $cPLA_2$ expression in HTSMCs was examined. As shown in Figure 1A, CSE-induced $cPLA_2$ expression was attenuated by pretreatment with tanshinone IIA (an AP-1 inhibitor) in a concentration-dependent manner. To further confirm the role of AP-1 in these responses, HTSMCs were transfected with siRNA of c-Jun or c-Fos for 24 h, serum-freed for 24 h, and then incubated with 50 μg/ml CSE for another 24 h. The expression of $cPLA_2$ protein was detected by Western blotting. As shown in Figure 1B, down-regulation of c-Jun or c-Fos protein expression by transfection with siRNA of either c-Jun or c-Fos reduced CSE-induced $cPLA_2$ expression in HTSMCs. In addition, CSE-stimulated $cPLA_2$ promoter activity and mRNA expression was inhibited by pretreatment with tanshinone IIA (Fig. 1C,D). These results suggested that CSE-induced $cPLA_2$ expression was, at least in part, mediated through AP-1 in HTSMCs.

CSE STIMULATES AP-1 EXPRESSION AND c-JUN PHOSPHORYLATION THROUGH NADPH OXIDASE/MAPKS PATHWAY

AP-1 is composed of c-Fos/c-Jun or c-Jun/c-Jun dimmers and is transcriptionally regulated by various stimuli [Vesely et al., 2009]. Thus, we investigated whether CSE could induce either c-Jun or c-Fos expression on HTSMCs. As shown in Figure 2A, CSE markedly stimulated both c-Jun and c-Fos protein expression and c-Jun phosphorylation in a time-dependent manner with a maximal response within 3 h and 30 min, respectively. It has been reported that up-regulation of c-Fos or c-Jun protein is mediated through activation of p42/p44 MAPK, p38 MAPK, and JNK1/2 [Lin et al., 2009; Tung et al., 2010]. Thus, whether activation of MAPKs contributed to the expression of AP-1 complex in CSE-stimulated HTSMCs was investigated. In this study, pretreatment with the inhibitor of MEK1/2 (U0126) or p38 MAPK (SB202190) significantly attenuated CSE-induced c-Fos mRNA and protein expression (Fig. 2B,C). However, pretreatment with SP600125 (an inhibitor of JNK1/2) only attenuated c-Jun mRNA and protein expression and c-Jun phosphorylation induced by CSE (Fig. 2B,C). Furthermore, transfection with siRNA encoding p42, JNK2, or p38 was applied to confirm these results. As shown in Figure 2D, transfection with either p42 siRNA or p38 siRNA inhibited CSE-induced c-Fos expression. On the other hand, transfection with JNK2 siRNA reduced CSE-induced c-Jun expression in HTSMCs. These results suggested that CSE-induced c-Fos expression is mediated through

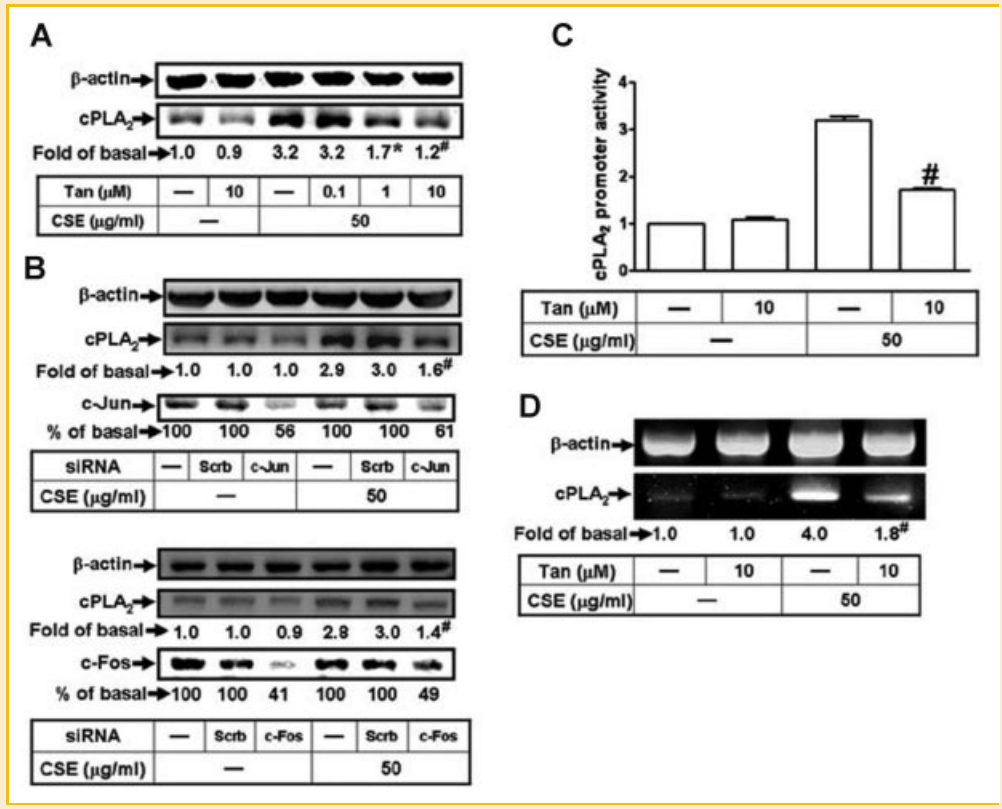

Fig. 1. CSE induces cPLA2 expression in HTSMCs via AP-1. A: HTSMCs were pretreated with tanshinone IIA for 1 h, and then incubated with 50 μg/ml of CSE for 24 h. The expression of cPLA2 was determined by Western blot. B: HTSMCs were transfected with siRNA of c-Jun, c-Fos or scrambled (Scrb), and then incubated with CSE (50 μg/ml) for 24 h. The expression of cPLA2, c-Jun, and c-Fos were determined by Western blot. C: Cells were transiently transfected with cPLA2-luc reporter gene, pretreated with tanshinone IIA for 1 h, and then incubated with CSE for 4 h. The promoter activity of cPLA2 was determined in the cell lysates. D: HTSMCs were pretreated with tanshinone IIA (10 μM) for 1 h, and then incubated with CSE (50 μg/ml) for 4 h. The RNA samples were analyzed by RT-PCR for the levels of cPLA2 mRNA expression. Data represent as mean ± SEM from three independent experiments (n = 3). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the cells exposed to CSE alone. The standard deviations are ±10% in each group (B).

p38 MAPK and p42/p44 MAPK and c-Jun expression through JNK1/2 in HTSMCs.

CSE-INDUCED AP-1 EXPRESSION THROUGH ACTIVATION OF NADPH OXIDASE AND ROS GENERATION

NADPH oxidase/ROS have been shown to regulate MAPKs and AP-1 activation which mediate CSE-induced $cPLA_2$ expression in HTSMCs [Cheng et al., 2009]. Thus, in this study, we further examined the roles of NADPH oxidase/ROS in CSE-induced c-Fos and c-Jun expression and activation. As shown in Figure 3A, pretreatment with the inhibitors of NADPH oxidase (APO and DPI) or a ROS scavenger (NAC) attenuated CSE-induced c-Jun and c-Fos expression and c-Jun phosphorylation. In addition, CSE-induced c-Jun and c-Fos mRNA levels was also reduced by pretreatment with these inhibitors (Fig. 3B). The phagocyte NADPH oxidase centers around a membrane-associated flavocytochrome b_{558} (composed of $gp91^{phox}$/NOX2 and $p22^{phox}$ subunits) which contains two hemes as well as binding sites for NADPH and FAD, and cytosolic components [Chowdhury et al., 2005; Abid et al., 2007]. Activated NADPH oxidase is a multimeric protein complex consisting of at least three cytosolic subunits of $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$. It has been demonstrated that $p47^{phox}$ predominates the complex translocation to membrane [Bedard and Krause, 2007]. Therefore, the roles of $p47^{phox}$ and NOX2 in CSE-induced c-Jun and c-Fos expression were investigated in HTSMCs transfected with either $p47^{phox}$ siRNA or NOX2 siRNA. As shown in Figure 3C, transfection with siRNA of either NOX2 or $p47^{phox}$ down-regulated the expression of respective proteins and markedly inhibited CSE-regulated c-Jun and c-Fos expression. These data suggested that CSE-induced c-Fos and c-Jun expression is mediated through activation of NADPH oxidase/ROS generation in HTSMCs.

CSE INDUCES $cPLA_2$ EXPRESSION VIA p300

CBP (CREB-binding protein) and p300 are versatile co-activators that link transcriptional activators to the basal transcriptional apparatus [Luo et al., 2008]. p300 has been shown to regulate the expression of $cPLA_2$ induced by IL-1β [Luo et al., 2008]. Therefore, the involvement of p300 in $cPLA_2$ expression induced by CSE was investigated using a pharmacological inhibitor of p300, garcinol. As shown in Figure 4A, pretreatment with garcinol for 1 h prior to CSE exposure caused an attenuation of $cPLA_2$ protein expression in a concentration-dependent manner. The involvement

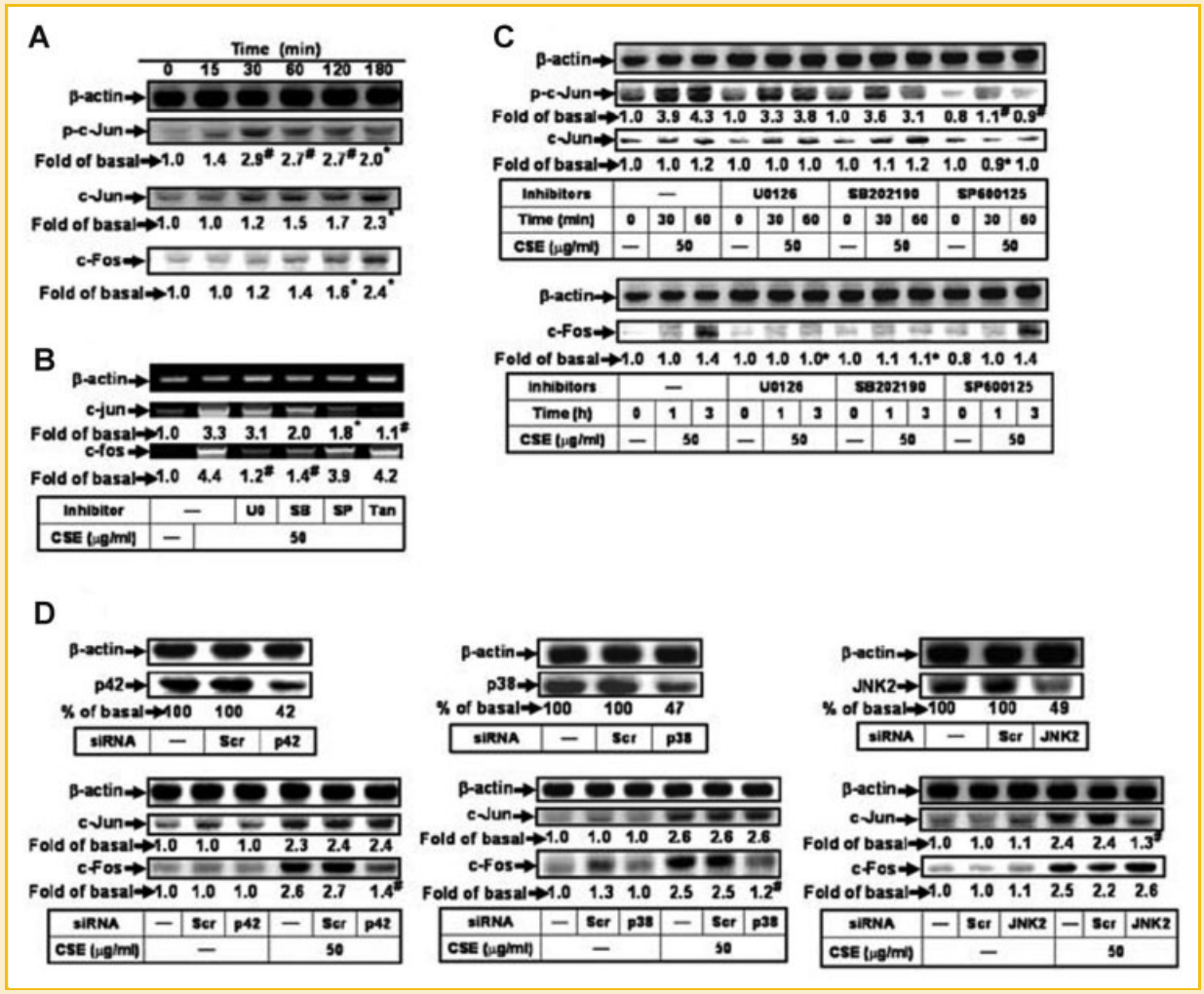

Fig. 2. CSE induces c-Jun and c-Fos expression via MAPKs in HTSMCs. A: Cells were incubated with CSE for the indicated time intervals. The levels of c-Jun and c-Fos expression and c-Jun phosphorylation were determined by Western blot using an anti-phospho-c-Jun, anti-c-Jun, anti-c- Fos, or anti-β-actin antibody. B: Cells were pretreated with U0126 (10 μM), SB202190 (10 μM), SP600125 (1 μM), or tanshinone IIA (10 μM) for 1 h, and then incubated with CSE for 15 min (for c-Jun) or 30 min (for c-Fos). The RNA samples were analyzed by RT-PCR for the levels of c-Jun and c-Fos mRNA. C: Cells were pretreated with U0126 (10 μM), SB202190 (10 μM), or SP600125 (1 μM) for 1 h, and then incubated with CSE for the indicated time intervals. The expression of c-Jun and c-Fos and c-Jun phosphorylation were determined by Western blot. D: Cells were transfected with siRNA of p42, p38, or JNK2, and then incubated with CSE for 3 h. The expression of c-Jun, c-Fos, p42, p38, and JNK2 were determined by Western blot. Data represent as mean of three independent experiments (n = 3). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the basal level (A) or the cells exposed to CSE alone (B,C).

of p300 in this response was further confirmed by transfection with p300 siRNA. As shown in Figure 4B, down-regulation of p300 protein expression by transfection with p300 siRNA markedly inhibited CSE-induced $cPLA_2$ expression in HTSMCs. In addition, pretreatment with garcinol also reduced CSE-stimulated $cPLA_2$ promoter activity (Fig. 4C). CS has been shown to regulate p300 activity in human breast cancer cells [Connors et al., 2009]. Thus, CSE-stimulated p300 phosphorylation was investigated in HTSMCs. We found that CSE stimulated p300 phosphorylation in a time-dependent manner with a maximal response within 2–3 h (Fig. 4D). Pretreatment with APO, DPI, NAC, or SP600125 significantly inhibited CSE-stimulated p300 phosphorylation in HTSMCs (Fig. 4E). These data showed that CSE-stimulated increase of $cPLA_2$ expression was mediated through an NADPH oxidase/ROS/JNK1/2/p300 pathway in HTSMCs.

NUCLEAR LOCALIZATION OF AP-1 BOUND TO $cPLA_2$ PROMOTER REGION

Further, we investigated whether CSE could stimulate the nuclear translocation of c-Jun and c-Fos in HTSMCs. We found that pretreatment with SP600125, APO, DPI, or NAC attenuated CSE-induced c-Jun nuclear translocation (Fig. 5A). On the other hand, CSE-induced c-Fos nuclear translocation was reduced by pretreatment with U0126, SB202190, APO, DPI, or NAC (Fig. 5A). The in vivo recruitment of c-Jun to the $cPLA_2$ promoter region was assessed by a ChIP assay. In vivo binding of c-Jun to the $cPLA_2$ promoter occurred as early as 15 min following CSE stimulation (Fig. 5B). The binding of c-Jun to the $cPLA_2$ promoter stimulated by CSE was attenuated by pretreatment with SP600125, APO, DPI, NAC, or tanshinone IIA (Fig. 5C). These results suggested that CSE-stimulated c-Jun or c-Fos nuclear translocation is mediated

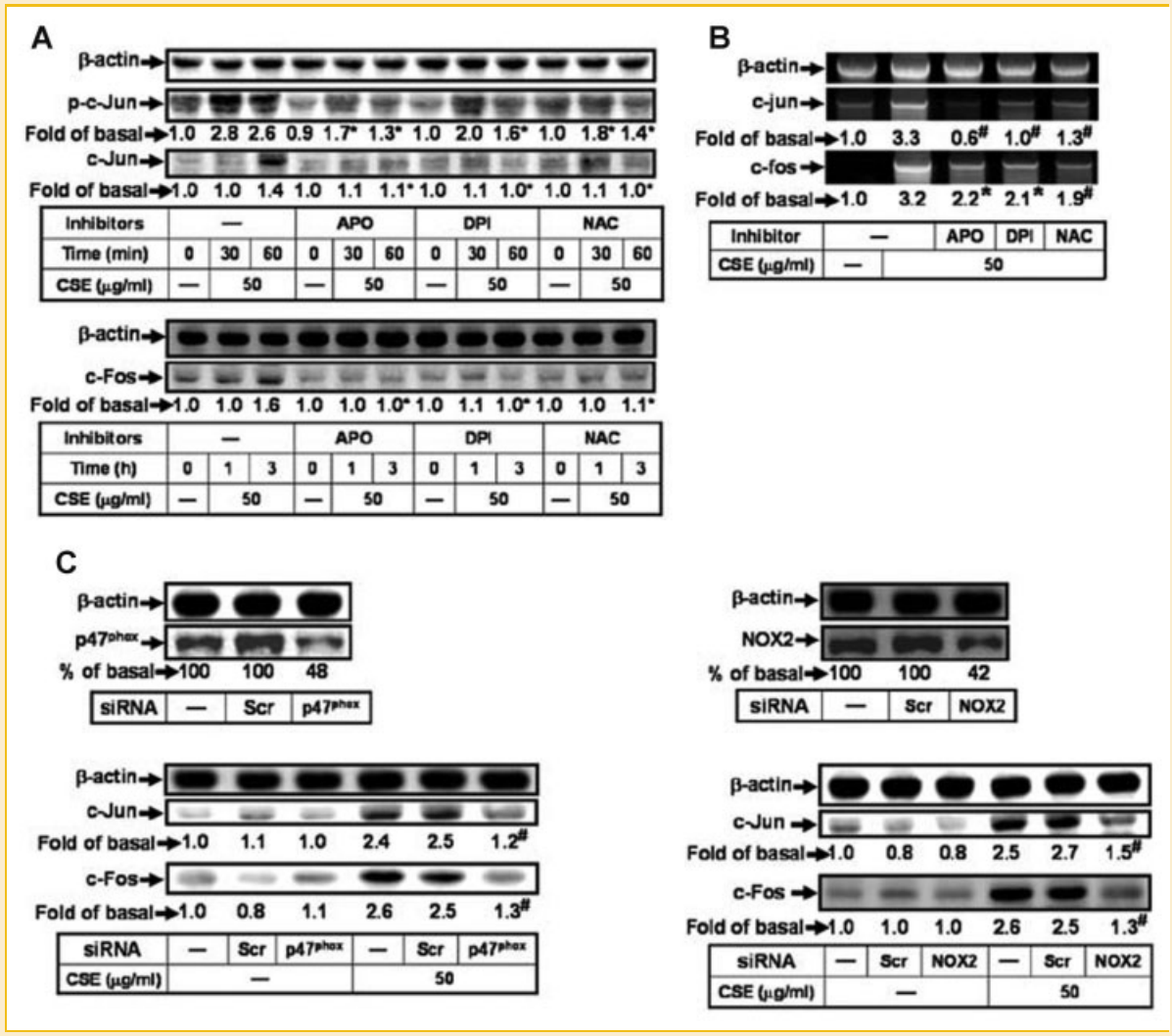

Fig. 3. CSE induces c-Jun and c-Fos expression via NADPH oxidase/ROS. A: Cells were pretreated with APO (100 μM), DPI (10 μM), or NAC (1 mM) for 1 h, and then incubated with CSE for the indicated time intervals. The expression of c-Jun and c-Fos and c-Jun phosphorylation were determined by Western blot. B: Cells were pretreated with APO (100 μM), DPI (10 μM), or NAC (1 mM) for 1 h, and then incubated with CSE for 15 min (for c-Jun) or 30 min (for c-Fos). The RNA samples were analyzed by RT-PCR for the levels of c-Jun and c-Fos mRNA. C: Cells were transfected with p47phox siRNA or NOX2 siRNA, and then treated with CSE for 3 h. The expression of c-Jun, c-Fos, p47phox, and NOX2 was determined by Western blot. Data represent as mean of three independent experiments ($n = 3$). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the cells exposed to CSE alone.

through activation of NADPH oxidase linking to JNK1/2 or p42/p44 MAPK and p38 MAPK, respectively, in HTSMCs.

CSE-INDUCED cPLA$_2$ EXPRESSION IS MEDIATED BY THE FORMATION OF A c-JUN/P300 COMPLEX

Previous studies have shown that the association between AP-1 and p300 leads to the enhancement of p300 transcriptional activity and increases the expression of proinflammatory genes [Nelson et al., 2006; Cascio et al., 2007]. Thus, we further investigated the physical association between c-Jun and p300 in CSE-induced cPLA$_2$ expression. As shown in Figure 6A, cells were incubated with CSE for the indicated time intervals, the cell lysates were subjected to immunoprecipitation using an anti-p300 or anti-c-Jun antibody, and then the immunoprecipitates were analyzed by Western blot using an anti-c-Jun or anti-p300 antibody. The protein levels of c-Jun and p300 were time-dependently increased in a p300- or c-Jun-immunoprecipitated complex, which were inhibited by pretreatment with APO, DPI, NAC, SP600125, or tanshinone IIA, but not with U0126 and SB202190 (Fig. 6B,C). The in vitro recruitment of p300 to the cPLA$_2$ promoter was assessed by a ChIP assay. The binding of p300 to the cPLA$_2$ promoter occurred as early as 15 min following CSE stimulation (Fig. 6D), which was attenuated by pretreatment with SP600125, APO, DPI, NAC, or tanshinone IIA (Fig. 6E). These results suggested that CSE-stimulated cPLA$_2$ expression is mediated via the formation of a c-Jun/p300 complex in HTSMCs.

DISCUSSION

CS has been shown to play an important role in the expression of many genes involved in various lung inflammatory diseases [Arredondo et al., 2008; Deshmukh et al., 2008]. Up-regulation of

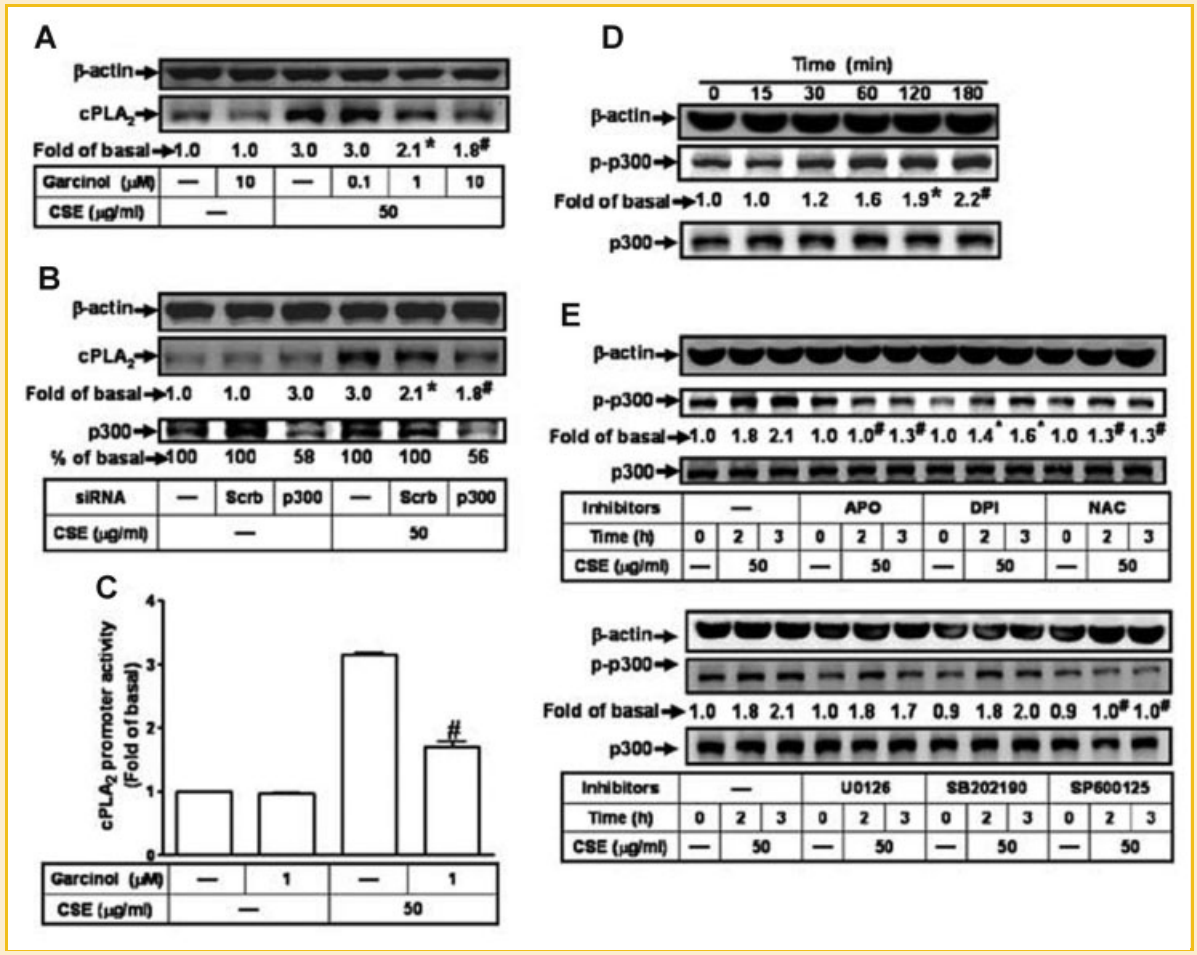

Fig. 4. CSE induces cPLA2 expression via p300 in HTSMCs. A: Cells were pretreated with garcinol for 1 h, and then incubated with CSE for 24 h. The expression of cPLA2 was determined by Western blot. B: Cells were transfected with p300 siRNA, and then incubated with CSE for 24 h. The expression of cPLA2 and p300 were determined by Western blot. C: Cells were transiently transfected with cPLA2-luc reporter gene, pretreated with garcinol, and then incubated with CSE for 4 h. The promoter activity of cPLA2 was determined. D: Cells were treated with CSE for the indicated time intervals. The cell lysates were subjected to Western blot using an anti-phospho p300 antibody. E: Cells were pretreated with APO (100 μM), DPI (10 μM), NAC (1 mM), U0126 (10 μM), SB202190 (10 μM), or SP600125 (1 μM) for 1 h, and then incubated with CSE for the indicted time intervals. The cell lysates were subjected to Western blot analysis. Data represent as mean of three independent experiments (n = 3). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the cells exposed to CSE alone (A–C,E) or with the basal level (D).

$cPLA_2$ expression by mesenchymal cells in several extra-pulmonary sites leads to generation of PGE_2, known as an important lipid mediator implicated in inflammatory responses [Khanapure et al., 2007]. Our previous study showed that CSE induced $cPLA_2$ expression via NADPH oxidase/ROS/MAPKs, AP-1 and NF-κB pathways in HTSMCs [Cheng et al., 2009]. CBP (CREB-binding protein) and p300 are versatile co-activators that link transcriptional activators to the basal transcriptional apparatus [Luo et al., 2008]. p300 has been shown to regulate the expression of $cPLA_2$ induced by IL-1β [Luo et al., 2008]. Thus, in this study, we investigated whether CSE induced $cPLA_2$ expression via AP-1 subunits (c-Fos and c-Jun) and p300 in HTSMCs. Here, by Western blot, RT-PCR, and promoter activity analyses coupled with pharmacological inhibitors and transfection with siRNAs revealed that CSE-induced $cPLA_2$ expression was mediated through NOX2-dependent p42/p44 MAPK and p38 MAPK/c-Fos and JNK1/2/c-Jun/p300 pathways in HTSMCs.

Many transcription factors like AP-1 and NF-κB have been shown to be involved in regulation of gene expression in asthmatic and COPD inflammation [Adcock et al., 2006]. AP-1 is a heterodimer of Fos and Jun oncoproteins which bind to the TPA (tetradecanoyl-phorbol-13-acetate) response element (TRE) [Barnes, 2006]. AP-1 is a collection of related transcription factors belonging to the Fos (c-Fos, FosB, Fra1, Fra2) and Jun (c-Jun, JunB, JunD) families, which dimerize in various combinations by formation of leucine zipper [Barnes, 2006]. Indeed, our previous study has shown that CSE induced $cPLA_2$ expression via AP-1 and NF-κB in HTSMCs [Cheng et al., 2009]. To further confirm the role of c-Fos and c-Jun in CSE-induced $cPLA_2$ expression, pretreatment with the inhibitor of AP-1 or transfection with siRNA of either c-Jun or c-Fos markedly attenuated CSE-induced $cPLA_2$ expression in HTSMCs.

AP-1 may be activated via PKCs, protein tyrosine kinases (PTK) and MAPKs by cytokines, which themselves activate a cascade of intracellular kinases [Barnes, 2006]. Certain signals including

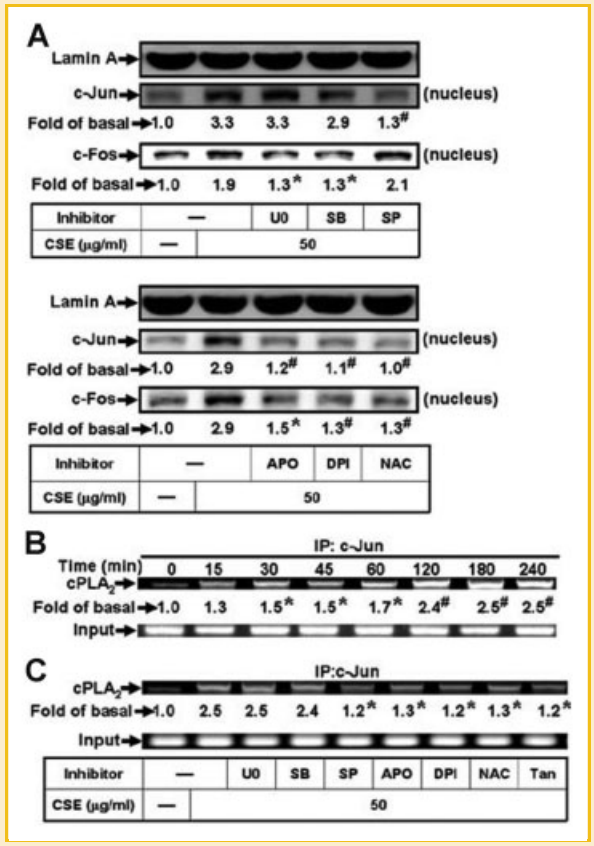

Fig. 5. Nuclear localization of AP-1 bound to cPLA2 promoter region. A: Cells were pretreated with U0126 (10 μM), SB202190 (10 μM), SP600125 (1 μM), APO (100 μM), DPI (10 μM), or NAC (1 mM) for 1 h, and then incubated with CSE for 1 h. The nuclear extracts were prepared and subjected to Western blot using an anti-c-Jun or anti-c-Fos antibody. B: An enrichment of the cPLA2 promoter DNA was shown after PCR amplification of immunoprecipitates of c-Jun-associated DNA from cells treated with CSE for the indicated time intervals. The ChIP assay was performed. C: Cells were pretreated with U0126 (10 μM), SB202190 (10 μM), SP600125 (1 μM), APO (100 μM), DPI (10 μM), NAC (1 mM), or tanshinone IIA (10 μM) for 1 h, and then incubated with CSE for 2 h. The ChIP assay was performed. Data represent as mean of three independent experiments (n = 3). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the basal level (B) or with the cells exposed to CSE alone (A,C).

MAPKs rapidly increase *c-fos* gene and protein expression and AP-1 activation in various cell types [Chien et al., 2009; Lin et al., 2009; Tung et al., 2010]. Thus, we investigated the roles of MAPKs in CSE-regulated c-Fos and c-Jun expression in HTSMCs. CSE markedly induced c-Jun and c-Fos expression and c-Jun phosphorylation through differential activation of MAPKs in these cells. We found that CSE-induced c-Jun protein expression and mRNA levels were inhibited by pretreatment with the inhibitors of JNK1/2 and AP-1. On the other hand, pretreatment with the inhibitors of MEK1/2 and p38 MAPK reduced CSE-induced c-Fos protein and mRNA expression. This note was further confirmed by transfection with siRNAs of p42 and p38, or JNK2 which specifically inhibited CSE-mediated c-Fos or c-Jun expression, respectively. These data suggested that p42/p44 MAPK, p38 MAPK, and JNK1/2 play differential roles in CSE-regulated c-Jun and c-Fos expression and activation. The detail mechanisms underlying these MAPKs regulated c-Jun and c-Fos activities are needed to be investigated in the future.

CS, allergens, or inflammatory mediator promotes free radicals production which plays an important role in the development of asthma and COPD. Oxidative stress occurs when the flux of ROS or free radical generation exceeds available antioxidant defenses [Foronjy et al., 2006]. The biological function of NADPH oxidase enzymes might be attributed to the production of ROS [Lagente et al., 2008]. In our previous study, CSE-induced $cPLA_2$ expression was mediated via NADPH oxidase/ROS in HTSMCs [Cheng et al., 2009]. The aim of the present study was to examine whether CSE-induced expression of c-Fos and c-Jun was mediated through NADPH oxidase activation. Indeed, our results showed that pretreatment with the NADPH oxidase inhibitors and a ROS scavenger suppressed CSE-induced c-Jun phosphorylation and c-Jun and c-Fos expression. Activated NADPH oxidase is a multimeric protein complex consisting of at least three cytosolic subunits of $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$. The $p47^{phox}$ regulatory subunit plays a critical role in activation of NADPH oxidase [Bedard and Krause, 2007]. Therefore, the involvement of $p47^{phox}$ in CSE-induced responses was confirmed by transfection with $p47^{phox}$ siRNA or NOX2 siRNA. Our results showed that transfection with these siRNAs inhibited CSE-induced c-Jun and c-Fos expression. Further, we also investigated whether CSE stimulated c-Jun and c-Fos expression in the nuclear fraction mediated through NADPH oxidase and MAPKs in HTSMCs. Pretreatment with the inhibitors of NADPH oxidase or a ROS scavenger attenuated CSE-induced c-Jun and c-Fos expression in nucleus and the binding of c-Jun to the $cPLA_2$ promoter. However, CSE-induced c-Jun translocation and the binding of c-Jun to the $cPLA_2$ promoter were inhibited by the inhibitor of JNK1/2, but not p38 MAPK and p42/p44 MAPK. These results suggested that CSE stimulated c-Jun translocation via an NADPH oxidase/JNK1/2-dependent manner.

The transcriptional co-activator p300 is a ubiquitous nuclear phosphoprotein and transcriptional cofactor with intrinsic acetyltransferase activity. p300 regulates the expression of numerous genes in a cell-type and signal-specific manner, and plays a pivotal role in cellular proliferation and apoptosis [Ghosh and Varga, 2007]. Accumulating evidence suggests that abnormal p300 activity is associated with inflammation, cancer, cardiac hypertrophy, and genetic disorders [Ghosh and Varga, 2007]. Thus, p300 involved in CSE-regulated $cPLA_2$ expression was investigated in HTSMCs. In this study, pretreatment with a p300 inhibitor or transfection with p300 siRNA reduced CSE-stimulated $cPLA_2$ protein levels and mRNA expression. Not surprisingly, the activity of p300 was regulated by post-translational modification including phosphorylation, methylation, acetylation, and sumoylation [Ghosh and Varga, 2007]. We also found that CSE stimulated p300 phosphorylation in HTSMCs. Site-specific p300 phosphorylation was catalyzed by various intracellular signal pathways, including MAPKs which resulted in structural and functional modifications and consequently regulated target gene expression [Ghosh and Varga, 2007]. Moreover, we also found that JNK1/2 was involved in CSE-induced p300 phosphorylation. On the other hand, pretreatment with the inhibitor of NADPH oxidase also inhibited CSE-

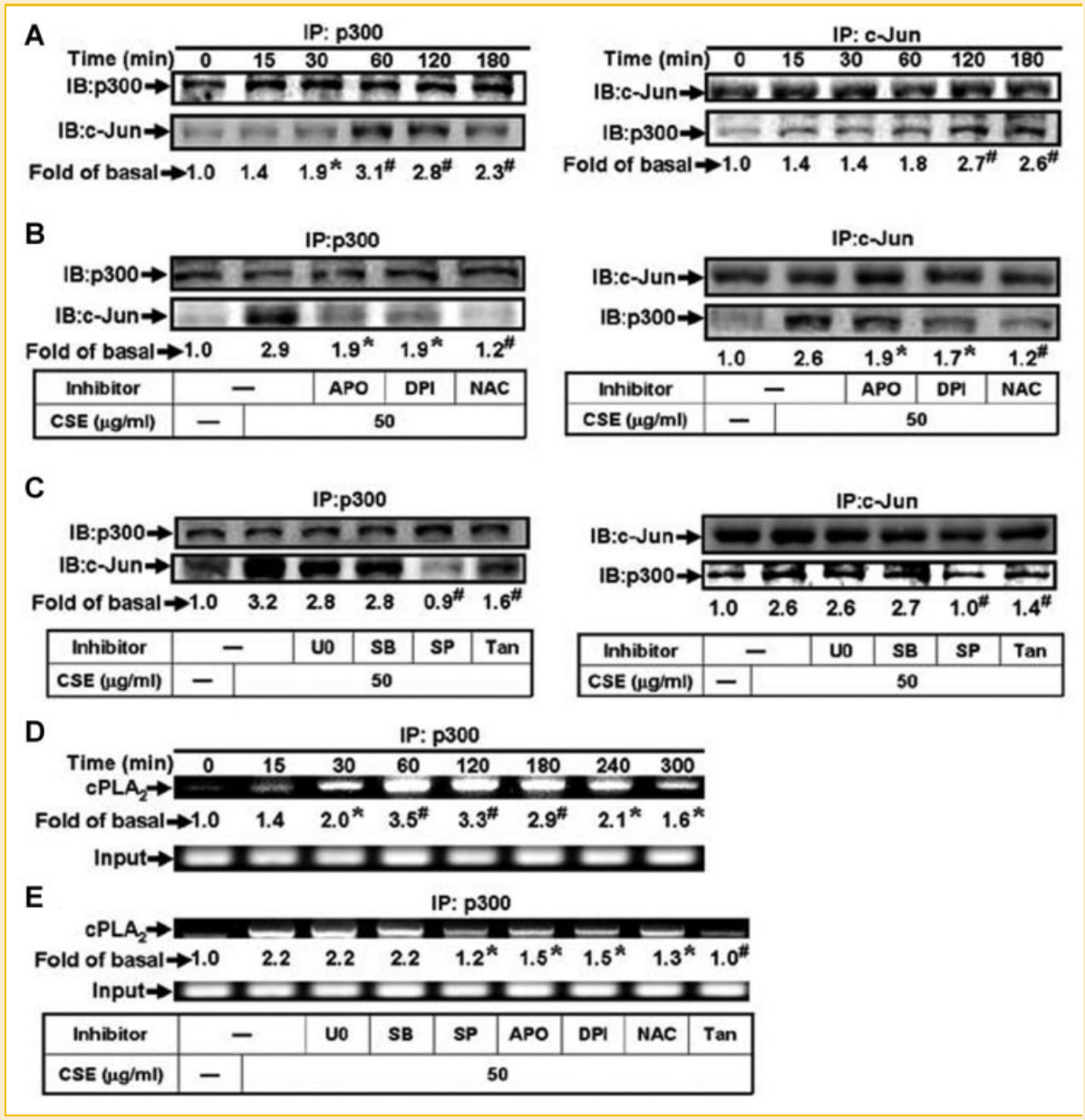

Fig. 6. CSE-enhanced cPLA2 expression is regulated by the formation of a c-Jun/p300 complex. A: Cells were incubated with CSE for the indicated time intervals. The cell lysates were subjected to immunoprecipitation using an anti-p300 or anti-c-Jun antibody, and then the immunoprecipitates were analyzed by Western blot using an anti-c-Jun or anti-p300 antibody. B,C: Cells were pretreated with APO (100 μM), DPI (10 μM), NAC (1 mM), U0126 (10 μM), SB202190 (10 μM), SP600125 (1 μM), or tanshinone IIA (10 μM) for 1 h, and then incubated with CSE for 2 h. The cell lysates were subjected to immunoprecipitation using an anti-p300 or anti-c-Jun antibody, and then the immunoprecipitates were analyzed by Western blot using an anti-c-Jun or anti-p300 antibody. D: An enrichment of the cPLA2 promoter DNA was shown after PCR amplification of immunoprecipitates of p300-associated DNA from the cells incubated with CSE for the indicated time intervals. The ChIP assay was performed. E: Cells were pretreated with U0126 (10 μM), SB202190 (10 μM), SP600125 (1 μM), APO (100 μM), DPI (10 μM), NAC (1 mM), or tanshinone IIA (10 μM) for 1 h, and then incubated with CSE for 2 h. The ChIP assay was performed. Data represent as mean of three independent experiments (n = 3). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the basal level (D). $^{*}P < 0.05$; $^{\#}P < 0.01$ as compared with the cells exposed to CSE alone (E).

induced p300 phosphorylation. These data showed that CSE induced p300 activation via NADPH oxidase/ROS/JNK1/2 in HTSMCs. Further, we found that NADPH oxidase and JNK1/2 regulated p300 activation and promoted association between p300 and c-Jun. Although the detailed protein–protein interactions between p300 and c-Jun are not known, our results show a novel role of c-Jun/p300 complex formation in CSE-induced $cPLA_2$ expression in HTSMCs. CSE-induced binding of p300 to the $cPLA_2$ promoter was inhibited by the inhibitors of NADPH oxidase and JNK1/2, but not p38 and p42/p44 MAPK. In the future, we will further determine which domains of c-Jun and p300 are involved in protein–protein interactions stimulated by CSE.

In conclusion, as depicted in Figure 7, our results showed that CSE induced c-Jun expression and phosphorylation or c-Fos expression

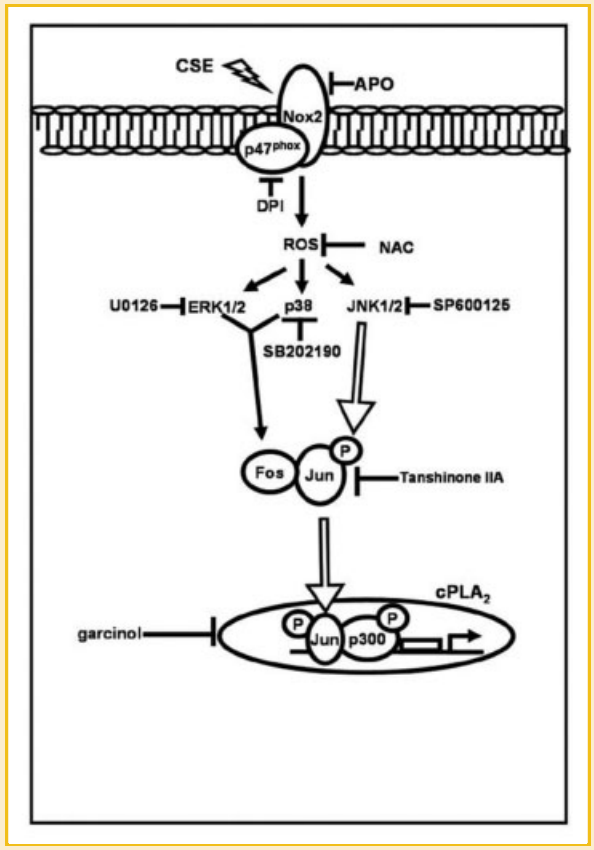

Fig. 7. Schematic diagram illustrating the proposed signaling pathways involved in CSE-induced cPLA2 expression in HTSMCs. CSE stimulates c-Jun phosphorylation via a NADPH oxidase/ROS/MAPK signaling, in turn initiates the activation of c-Fos and c-Jun. MAPKs also enhances p300 phosphorylation. Activated AP-1 and p300 are recruited to the promoter region of cPLA2 leading to an increase of cPLA2 expression.

via a NADPH oxidase-dependent JNK1/2 or p42/p44 MAPK and p38 MAPK, in turn initiated the activation of AP-1 and p300. Activated AP-1 and p300 were recruited to the promoter region of $cPLA_2$ leading to an increase of $cPLA_2$ expression in HTSMCs.

ACKNOWLEDGMENTS

This work was supported by grants NSC98-2314-B-182-021-MY3 (CCL), NSC96-2320-B-182-003, and NSC96-2320-B-182-047-MY3 from National Science Council, Taiwan and CMRPD32043, CMRPD170332, CMRPG381522, CMRPG350653, and CMRPG360513 from Chang Gung Medical Research Foundation, Taiwan. We thank Mr. Li-Der Hsiao for his technical assistance during preparation of this manuscript.

REFERENCES

Abid MR, Spokes KC, Shih SC, Aird WC. 2007. NADPH oxidase activity selectively modulates vascular endothelial growth factor signaling pathways. J Biol Chem 282:35373–35385.

Adcock IM, Chung KF, Caramori G, Ito K. 2006. Kinase inhibitors and airway inflammation. Eur J Pharmacol 533:118–132.

Arredondo J, Chernyavsky AI, Jolkovsky DL, Pinkerton KE, Grando SA. 2008. Receptor-mediated tobacco toxicity: Acceleration of sequential expression of α5 and α7 nicotinic receptor subunits in oral keratinocytes exposed to cigarette smoke. FASEB J 22:1356–1368.

Balsinde J, Balboa MA, Insel PA, Dennis EA. 1999. Regulation and inhibition of phospholipase A_2. Annu Rev Pharmacol Toxicol 39:175–189.

Barnes PJ. 1989. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. J Allergy Clin Immunol 83:1013–1026.

Barnes PJ. 2006. Transcription factors in airway diseases. Lab Invest 86:867–872.

Bedard K, Krause KH. 2007. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. Physiol Rev 87:245–313.

Bonventre JV, Sapirstein A. 2002. Group IV cytosolic phospholipase A_2 (PLA_2) function: Insights from the knockout mouse. Adv Exp Med Biol 507:25–31.

Cascio S, Bartella V, Garofalo C, Russo A, Giordano A, Surmacz E. 2007. Insulin-like growth factor 1 differentially regulates estrogen receptor-dependent transcription at estrogen response element and AP-1 sites in breast cancer cells. J Biol Chem 282:3498–3506.

Cheng SE, Luo SF, Jou MJ, Lin CC, Kou YR, Lee IT, Hsieh HL, Yang CM. 2009. Cigarette smoke extract induces cytosolic phospholipase A_2 expression via NADPH oxidase, MAPKs, AP-1, and NF-κB in human tracheal smooth muscle cells. Free Radic Biol Med 46:948–960.

Chien MH, Ku CC, Johansson G, Chen MW, Hsiao M, Su JL, Inoue H, Hua KT, Wei LH, Kuo ML. 2009. Vascular endothelial growth factor-C (VEGF-C) promotes angiogenesis by induction of COX-2 in leukemic cells via the VEGF-R_3/JNK/AP-1 pathway. Carcinogenesis 30:2005–2013.

Chowdhury AK, Watkins T, Parinandi NL, Saatian B, Kleinberg ME, Usatyuk PV, Natarajan V. 2005. Src-mediated tyrosine phosphorylation of $p47^{phox}$ in hyperoxia-induced activation of NADPH oxidase and generation of reactive oxygen species in lung endothelial cells. J Biol Chem 280:20700–20711.

Connors SK, Balusu R, Kundu CN, Jaiswal AS, Gairola CG, Narayan S. 2009. C/EBPβ-mediated transcriptional regulation of bcl-xl gene expression in human breast epithelial cells in response to cigarette smoke condensate. Oncogene 28:921–932.

Deshmukh HS, Shaver C, Case LM, Dietsch M, Wesselkamper SC, Hardie WD, Korfhagen TR, Corradi M, Nadel JA, Borchers MT, Leikauf GD. 2008. Acrolein-activated matrix metalloproteinase 9 contributes to persistent mucin production. Am J Respir Cell Mol Biol 38:446–454.

DeWitt DL. 1999. Cox-2-selective inhibitors: The new super aspirins. Mol Pharmacol 55:625–631.

Dieter P, Kolada A, Kamionka S, Schadow A, Kaszkin M. 2002. Lipopolysaccharide-induced release of arachidonic acid and prostaglandins in liver macrophages: Regulation by Group IV cytosolic phospholipase A_2, but not by Group V and Group IIA secretory phospholipase A_2. Cell Signal 14:199–204.

Foronjy RF, Mirochnitchenko O, Propokenko O, Lemaitre V, Jia Y, Inouye M, Okada Y, D'Armiento JM. 2006. Superoxide dismutase expression attenuates cigarette smoke- or elastase-generated emphysema in mice. Am J Respir Crit Care Med 173:623–631.

Ghosh AK, Varga J. 2007. The transcriptional coactivator and acetyltransferase p300 in fibroblast biology and fibrosis. J Cell Physiol 213:663–671.

Ghosh M, Stewart A, Tucker DE, Bonventre JV, Murphy RC, Leslie CC. 2004. Role of cytosolic phospholipase A_2 in prostaglandin E_2 production by lung fibroblasts. Am J Respir Cell Mol Biol 30:91–100.

Henderson WR, Jr., Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, Jonas M, Pae C, Wang H, Chi EY. 2002. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. Am J Respir Crit Care Med 165:108–116.

Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, Herszberg B, Lavoie JP, McVicker CG, Moir LM, Nguyen TT, Peng Q, Ramos-Barbon D,

Stewart AG. 2004. Proliferative aspects of airway smooth muscle. J Allergy Clin Immunol 114:S2–S17.

Khanapure SP, Garvey DS, Janero DR, Letts LG. 2007. Eicosanoids in inflammation: Biosynthesis, pharmacology, and therapeutic frontiers. Curr Top Med Chem 7:311–340.

Lagente V, Planquois JM, Leclerc O, Schmidlin F, Bertrand CP. 2008. Oxidative stress is an important component of airway inflammation in mice exposed to cigarette smoke or lipopolysaccharide. Clin Exp Pharmacol Physiol 35:601–605.

Lee IT, Wang SW, Lee CW, Chang CC, Lin CC, Luo SF, Yang CM. 2008. Lipoteichoic acid induces HO-1 expression via the TLR2/MyD88/c-Src/NADPH oxidase pathway and Nrf2 in human tracheal smooth muscle cells. J Immunol 181:5098–5110.

Leslie CC. 1997. Properties and regulation of cytosolic phospholipase A_2. J Biol Chem 272:16709–16712.

Lin CC, Kuo CT, Cheng CY, Wu CY, Lee CW, Hsieh HL, Lee IT, Yang CM. 2009. IL-1β promotes A549 cell migration via MAPKs/AP-1- and NF-κB-dependent matrix metalloproteinase-9 expression. Cell Signal 21:1652–1662.

Luo SF, Lin CC, Chen HC, Lin WN, Lee IT, Lee CW, Hsiao LD, Yang CM. 2008. Involvement of MAPKs, NF-κB and p300 co-activator in IL-1β-induced cytosolic phospholipase A_2 expression in canine tracheal smooth muscle cells. Toxicol Appl Pharmacol 232:396–407.

Martey CA, Baglole CJ, Gasiewicz TA, Sime PJ, Phipps RP. 2005. The aryl hydrocarbon receptor is a regulator of cigarette smoke induction of the cyclooxygenase and prostaglandin pathways in human lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 289:L391–L399.

Moodie FM, Marwick JA, Anderson CS, Szulakowski P, Biswas SK, Bauter MR, Kilty I, Rahman I. 2004. Oxidative stress and cigarette smoke alter chromatin remodeling but differentially regulate NF-κB activation and proinflammatory cytokine release in alveolar epithelial cells. FASEB J 18: 1897–1899.

Morri H, Ozaki M, Watanabe Y. 1994. 5′-Flanking region surrounding a human cytosolic phospholipase A_2 gene. Biochem Biophys Res Commun 205:6–11.

Mur C, Claria J, Rodela S, Lario S, Campistol JM, Titos E, Inigo P, Cases A, Abian J, Esmatjes E. 2004. Cigarette smoke concentrate increases 8-epi-$PGF_{2\alpha}$ and TGFβ1 secretion in rat mesangial cells. Life Sci 75:611–621.

Nagaraj NS, Zacharias W. 2007. Cigarette smoke condensate increases cathepsin-mediated invasiveness of oral carcinoma cells. Toxicol Lett 170:134–145.

Nagase T, Uozumi N, Ishii S, Kume K, Izumi T, Ouchi Y, Shimizu T. 2000. Acute lung injury by sepsis and acid aspiration: A key role for cytosolic phospholipase A_2. Nat Immunol 1:42–46.

Nelson KK, Subbaram S, Connor KM, Dasgupta J, Ha XF, Meng TC, Tonks NK, Melendez JA. 2006. Redox-dependent matrix metalloproteinase-1 expression is regulated by JNK through Ets and AP-1 promoter motifs. J Biol Chem 281:14100–14110.

Nie M, Pang L, Inoue H, Knox AJ. 2003. Transcriptional regulation of cyclooxygenase 2 by bradykinin and interleukin-1β in human airway smooth muscle cells: Involvement of different promoter elements, transcription factors, and histone h4 acetylation. Mol Cell Biol 23:9233–9244.

Rahman I, Adcock IM. 2006. Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J 28:219–242.

Ramage L, Jones AC, Whelan CJ. 2006. Induction of apoptosis with tobacco smoke and related products in A549 lung epithelial cells in vitro. J Inflamm (Lond) 3:3.

Six DA, Dennis EA. 2000. The expanding superfamily of phospholipase A_2 enzymes: Classification and characterization. Biochim Biophys Acta 1488: 1–19.

Tung WH, Hsieh HL, Yang CM. 2010. Enterovirus 71 induces COX-2 expression via MAPKs, NF-κB, and AP-1 in SK-N-SH cells: Role of PGE_2 in viral replication. Cell Signal 22:234–246.

Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y, Miyazaki J, Shimizu T. 1997. Role of cytosolic phospholipase A_2 in allergic response and parturition. Nature 390:618–622.

Vesely PW, Staber PB, Hoefler G, Kenner L. 2009. Translational regulation mechanisms of AP-1 proteins. Mutat Res 682:7–12.

Yang CM, Chien CS, Hsiao LD, Luo SF, Wang CC. 2002. Interleukin-1beta-induced cyclooxygenase-2 expression is mediated through activation of p42/44 and p38 MAPKS, and NF-κB pathways in canine tracheal smooth muscle cells. Cell Signal 14:899–911.

Zenz R, Eferl R, Scheinecker C, Redlich K, Smolen J, Schonthaler HB, Kenner L, Tschachler E, Wagner EF. 2008. Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. Arthritis Res Ther 10:201.