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Activation of nuclear factor kappa B by diesel exhaust particles in mouse epidermal cells through phosphatidylinositol 3-kinase/Akt signaling pathway

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Abstract

Diesel exhaust particles (DEP) induce intense inflammatory and allergic immune responses. The epidermal cells receive much exposure to DEP, and are an important source of pro-inflammatory cytokines and other inflammatory mediators. Transcription factors, such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), regulate the expression of these mediators. We hypothesize that the transcription factors are target of DEP action. The current study sought to determine whether DEP-activated NF-κB and AP-1 in a mouse epidermal cell line, JB6 P^+ cells. Using stable transfectants of JB6 P^+ cells expressing NF-κB or AP-1 luciferase reporter constructs, we demonstrated that exposure to DEP at a non-cytotoxic concentration significantly enhanced the transactivation of NF-κB, but not AP-1. Furthermore, DEP promoted phosphorylation of Akt, a substrate of phosphatidylinositol 3-kinase (PI3K), on Ser-473 and Thr-308 in a PI3K-dependent manner, and enhanced phosphorylation of down-stream p70/p85 S6 kinases (p70/p85S6K) as well as glycogen synthase kinase-3β (GSK-3β). Blockage of PI3K activation eliminated DEP-stimulated NF-κB transactivation. Although SAPK/JNK pathway was modestly activated by DEP, it was not involved in NF-κB transactivation. DEP had little effect on the phosphorylation of ERKs and p38 MAPK. Thus, DEP-induced transactivation of NF-κB is mediated by PI3K/Akt signaling pathway.

Keywords: Air pollutants; Cytokines; Inflammation; Signal transduction; Skin; Transcription factors

1. Introduction

Air pollution poses a severe risk for human health in industrialized countries. Both gaseous and particulate emissions resulting from motor vehicle traffic contribute to this phenomenon. Diesel exhaust particles (DEP) are among the most abundant components of particulate matter with an aerodynamic diameter <2.5 μm (PM2.5). Epidemiological and experimental studies indicate that DEP are capable of inducing intense inflammatory reaction and allergic immune response [1–4].

DEP-induced inflammatory changes in airways and development of various lung diseases have been well documented [1,3,5]. Exposure to DEP results in an increase in the production of immunoglobulin (IgE) and pro-inflammatory cytokine in various types of airway cells, including epithelial cells [4,6–11]. It is believed that the elevated levels of these inflammatory mediators trigger inflammatory response and allergic diseases during repeated exposure to DEP. DEP-induced up-regulation of cytokines occurs at non-cytotoxic concentrations and is likely mediated by gene transcription [4,12].

The epidermis is a rich source of cytokines and growth factors [13,14]. Like airway epithelial cells, keratinocytes, the major cell mass of the epidermis, not only represents the first target of irritants but may also act as a 'signal transducer,' capable of converting exogenous stimuli into the production of cytokines, adhesion molecules

Abbreviations: AP-1, activator protein 1; DEP, diesel exhaust particles; GSK-3β, glycogen synthase kinase-3β; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; p70S6K, p70 S6 kinase; PI3K, phosphatidylinositol 3-kinase

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and chemotactic factors [15]. It has been shown that keratinocytes play an important role in the pathogenesis of cutaneous inflammatory disease by producing pro-inflammatory cytokines such as interleukin 1α (IL-1α), IL-1β, IL-6, IL-8, tumor necrosis factor α (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [16]. DEP have been shown to induce the production of pro-inflammatory cytokines by human dermal keratinocytes and are suggested to be involved in the initiation or pathogenesis of allergic or non-allergic cutaneous inflammation [17]. Gene transcription of pro-inflammatory cytokines is regulated by transcription factors. Nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) are two important and well-documented transcription factors that regulate the transcription of these cytokines. The present study is designed to determine whether DEP activate NF-κB and AP-1 in a mouse epidermal cell line (JB6 P^+) and to further delineate signaling pathways that mediate the activation. JB6 P^+ cells, originally derived from primary mouse epidermal cells, are able to undergo transformation in response to 12-*O*-tetradecanoylphorbol 13-acetate or epidermal growth factor [18].

2. Materials and methods

2.1. Materials

The DEP extract was purchased from the National Institute of Standards and Technology [standard reference material # 1975 (SRM 1975)]. This sample had a mass median aerodynamic diameter of approximately 0.5 μm. The DEP extract was dissolved in DMSO at a stock concentration of 20 mg/ml. Other chemicals of the highest grade available were obtained from Sigma Chemical Co. unless otherwise mentioned. All antibodies except anti-actin were obtained from Cell Signaling Technology, Inc. Anti-actin was purchased from Santa Cruz Biotechnology. MEK1 inhibitor (PD98059), p38 MAPK inhibitor (SB203580) and Synthetic GSK3β inhibitor (TDZD-8) were obtained from Calbiochem. PI3K inhibitor (LY294002 and wortmannin) was purchased from Promega. JNK inhibitor (D-JNKI1) was obtained from Alexis Biochemicals.

2.2. Culture and treatment of mouse epidermal cell lines

JB6 P^+ mouse epidermal cell line (Cl 41) was grown in Eagle's MEM containing 10% fetal bovine serum (FBS), 2 mM L-glutamine and 25 μg/ml gentamicin at 37 °C with 5% CO_2. The stable transfectants of JB6 P^+ Cl 41 cells expressing NF-κB or AP-1-luciferase reporter (Cl 41 AP-1) and dominant-negative PI3K regulatory p85 subunit ($JB6^{DNp85}$) have been previously described [19,20]. Cells were exposed to DEP (5-160 μg/ml) for the specified periods. For blocking intracellular kinase activity, cells were treated with selective protein kinase inhibitors 30 min prior to DEP exposure.

2.3. MTT assay

The MTT assay was employed to determine the number of viable cells in culture (Roche Molecular Biochemicals) [21]. The assay is based on the cleavage of the yellow tetrazolium salt MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] to form the purple formazan crystals by metabolically active cells. Briefly, the cells were plated into 96-well microtiter plates and exposed to either DEP (5–160 μg/ml) or DMSO (control) for 48 h. Following DEP exposure, 10 μl of MTT labeling reagent were added to each well and the plates were incubated at 37 °C for 4 h. The cultures were then solubilized and spectrophotometric absorbance of the samples was detected by a microtiter plate reader. The wavelength to measure absorbance of formazan product is 570 nm, with a reference wavelength of 750 nm.

2.4. Measurement of NF-κB and AP-1 activity

NF-κB and AP-1 transactivation in JB6 P^+ epidermal cells was determined by assaying the activity of the luciferase reporter [19,20]. Briefly, cells were cultured in 96-well plates and grown in a medium containing 10% FBS. The plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2. For assaying NF-κB or AP-1 activity, sub-confluent cultures were maintained in a medium containing 0.1% FBS for 24 h and subsequently treated with DEP or TPA for the specified durations. After treatment, cellular protein was extracted with a 1× lysis buffer supplied in the luciferase assay kit (Promega), and luciferase activity was measured with a luminometer (3010, Analytical Luminescence Laboratory). NF-κB or AP-1 activity (luciferase activity) was calculated and expressed relative to the untreated cultures.

2.5. Immunoblotting

The immunoblotting procedure for detecting phosphorylation and expression of signal proteins was performed as previously described [22]. Briefly, cells were washed with phosphate-buffered saline (PBS, pH 7.4) and lysed with RIPA buffer [150 mM NaCl, 50 mM Tris pH 8.0, 1% Nonidet P-40, 0.1% sodium dodecylsulfate (SDS), 0.5% deoxycholic acid sodium, 0.1 mg/ml phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 3% Aprotinin (Sigma)] for 10 min on ice. The cell lysates were centrifuged, the supernatant was collected, and the protein concentration was determined. Aliquots of the protein (40 μg) were loaded onto the lanes of a SDS 10% polyacrylamide gel. The proteins were separated by electrophoresis, and the separated proteins were transferred to nitrocellulose membranes. The membranes were blocked

with either 5% non-fat dry milk or 5% BSA (for detection of phosphorylation) in 0.010 M PBS (pH 7.4) and 0.05% Tween-20 (TPBS) at room temperature for 1 h to block non-specific immunoreactivity. Subsequently, the membranes were incubated with primary antibodies directed against signal proteins for 1.5 h at room temperature or overnight at 4 °C. After three quick washes in TPBS, the membranes were incubated with a secondary antibody conjugated to horseradish peroxidase (Amersham) diluted at 1:2000 in TPBS for 1 h. The immune complexes were detected by the enhanced chemiluminescence method (Amersham). In some cases, the blots were stripped and re-probed with an anti-actin antibody (Santa Cruz Biotech).

2.6. Statistical analysis

Differences among treatment groups were tested using analysis of variance (ANOVA). Differences in which P was less than 0.05 were considered statistically significant. In cases where significant differences were detected, specific post hoc comparisons between treatment groups were examined with Student–Newman–Keuls tests.

3. Results

3.1. Effect of DEP on the viability of JB6 P^+ cells

MTT assay was performed to determine the cytotoxic concentration of DEP. As shown in Fig. 1A, at the concentration of 20 μg/ml or less, DEP did not significantly affect the viability of JB6 P^+ cells cultured in a serum-free medium; however, at a high concentration (>20 μg/ml), DEP caused cell death. A similar result was observed in cells cultured in a medium containing 10% FBS (Fig. 1B). In all the subsequent studies, therefore, a non-cytotoxic concentration of DEP (5–20 μg/ml) was used.

3.2. Effect of DEP on the activation of NF-κB and AP-1

As shown in Fig. 2, DEP significantly stimulated NF-κB activity at the concentrations of 5 and 10 μg/ml. Although its stimulatory effect was still statistically significant, DEP-induced activation of NF-κB was decreased at 20 μg/ml. However, DEP did not affect AP-1 activity. As a positive control, TPA significantly increased AP-1 activity by three-folds (Fig. 2C). Study of time sequence demonstrated that the maximal activation of NF-κB occurred at 6–12 h following DEP treatment. Again, at each time point examined, DEP did not alter the AP-1 activity.

3.3. Effect of DEP on PI3K/Akt signaling pathway

As shown in Fig. 3A, DEP-induced phosphorylation of Akt on both Thr-308 and Ser-473. Since phosphorylation of Akt can be independent of PI3K [23], we further investigated whether the activation of Akt was mediated by PI3K. First, we showed that LY294002 (10 μM), a specific inhibitor of PI3K, effectively blocked the phosphorylation of Akt, p70/p85 S6 kinase and GSK-3β (Fig. 3B). We further demonstrated that LY294002 eliminated DEP-mediated phosphorylation of Akt (Fig. 3C). In addition, inhibition of PI3K activation by expressing

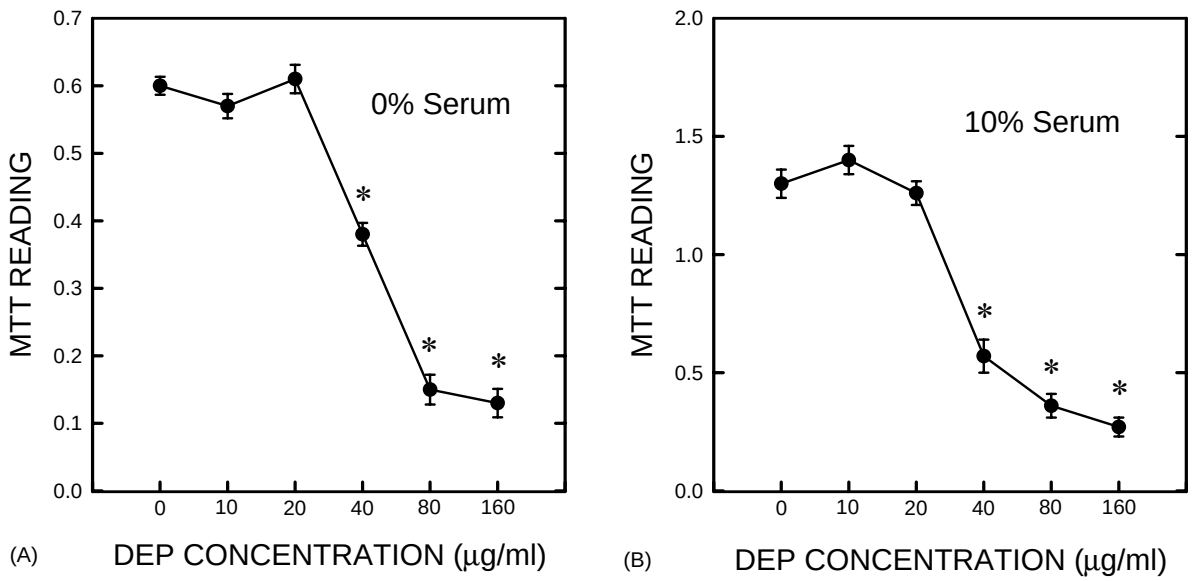

Fig. 1. Effect of DEP on the viability of JB6 P^+ cells. Cells were grown in either a serum-free medium (A) or a medium containing 10% FBS (B) and exposed to DEP (0–160 μg/ml) for 48 h. DEP was dissolved in DMSO and the control cultures were exposed to DMSO. The viability of cells was determined by MTT assay as described in Section 2. Each data point (±S.E.M.; bars) is the mean of five replicates. Asterisk (*) denotes a statistically significant difference from controls ($P < 0.05$).

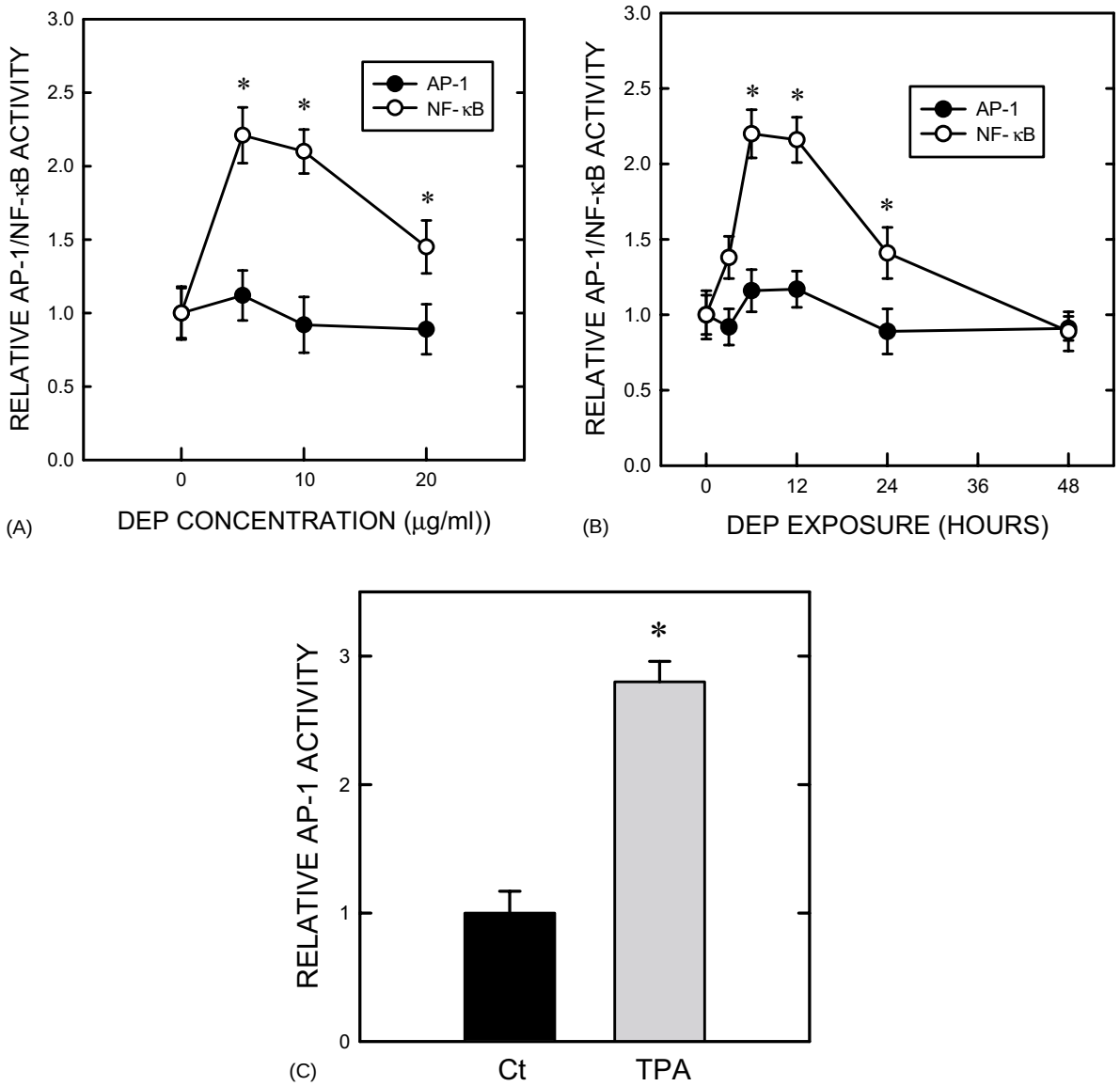

Fig. 2. Effect of DEP on NF-κB and AP-1 activity. (A) Dose-dependent effect of DEP. JB6 P^+ cells stably expressing NF-κB and AP-1-luciferase reporter constructs were exposed to DEP (0–20 μg/ml) for 12 h. The control cultures were exposed to DMSO. The relative activity of NF-κB and AP-1 (luciferase activity) was determined as described in Section 2. (B) Time sequence of DEP action. JB6 P^+ cells stably expressing NF-κB and AP-1-luciferase reporter were exposed to DEP (10 μg/ml) for 0–48 h. The relative activity of NF-κB and AP-1 was determined. (C) Effect of TPA on AP-1 activation. JB6 P^+ cells stably expressing AP-1-luciferase reporter construct were exposed to TPA (20 ng/ml) for 12 h, and AP-1 activity was determined. Each data point (±S.E.M.; bars) is the mean of four replicates. Asterisk (*) denotes a statistically significant difference from controls ($P < 0.05$).

dominant-negative p85 (regulatory subunit of PI3K) also blocked DEP-induced Akt phosphorylation (Fig. 3D). It was noted the basal phosphorylation of Akt was high in $JB6^{DNp85}$ cells. This could be due to a compensatory response to the constitutive inhibition of PI3K, because Akt phosphorylation can be regulated by pathways independent of PI3K [23]. DEP did not alter either the phosphorylation or expression of phosphatase and tensin homologue deleted on chromosome-10 (PTEN) (Fig. 3A), a dual specificity phosphatase that dephosphorylates PtdIns(3,4,5)P_3, suggesting that the effect of DEP was not mediated by the inhibition of dephosphorylation.

DEP exposure induced phosphorylation GSK-3β on Ser-9 (Fig. 4) without significantly affecting tyrosine (Tyr-216) phosphorylation (data not shown). The antibody directed against phospho-p70S6K (Thr-421/Ser-424) (Cell Signaling Inc.) also reacted with phospho-p85S6K. DEP drastically enhanced phosphorylation of p70/p85S6K on Thr-421/Ser-424, but only induced a modest increase in the phosphorylation of p70/p85S6K on Thr-389 (Fig. 4). The antibody directed against p70S6K also reacts with phosphorylated PKC-α. However, DEP exposure did not affect the phosporylation of FKHR, a distal component of PI3K/Akt signaling

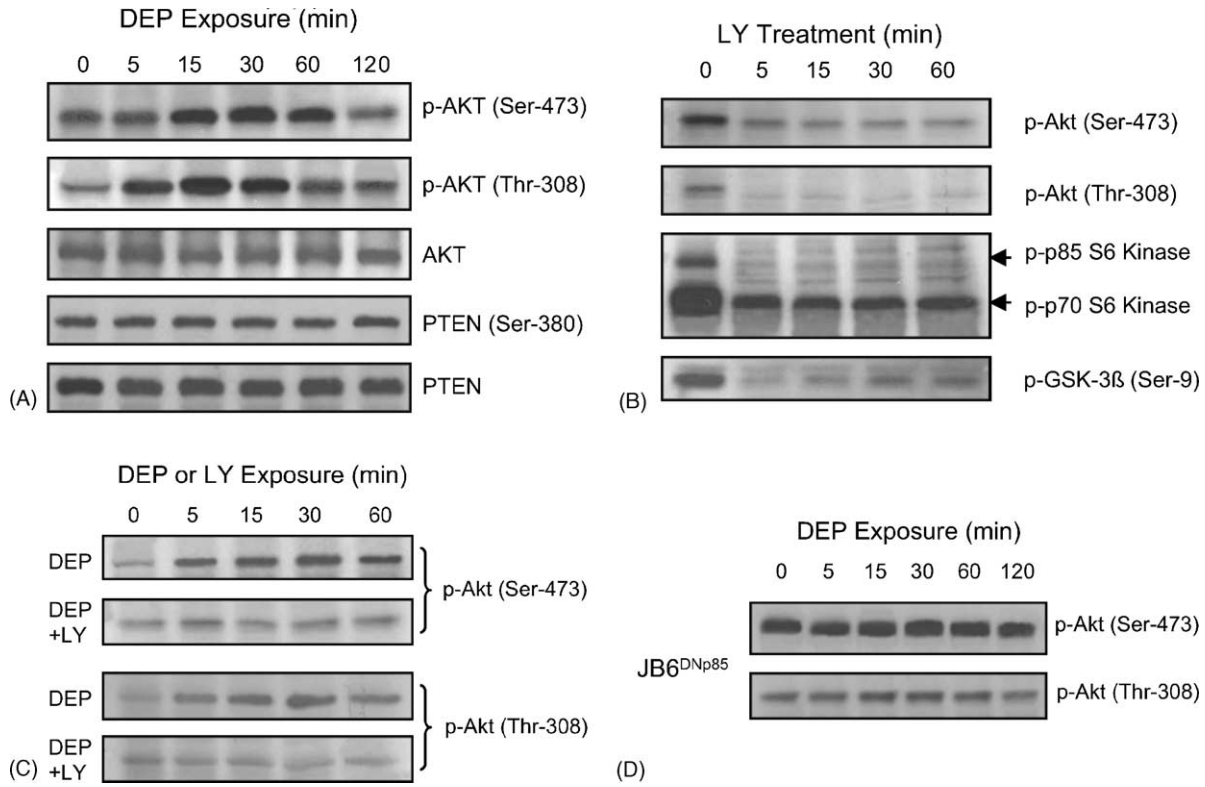

Fig. 3. Effect of DEP on phosphorylation of Akt. (A) The phosphorylation and expression of Akt and PTEN. JB6 P^+ cells were grown in a serum-free medium, and exposed to DEP (10 μg/ml, 5–120 min). Phosphorylation and expression of Akt and PTEN were determined with immunoblot using specific antibodies directed against either phosphorylated or regular forms of Akt or PTEN as described in Section 2. (B) Effect of LY294002 on the phosphorylation of Akt, p70/p85 S6 kinase and GSK-3β. JB6 P^+ cells were grown in a medium containing 5% FBS and treated with LY294002 (10 μM) for specified period. Phosphorylation of phosphorylation of Akt, p70/p85 S6 kinase and GSK-3β was determined with immunoblot using specific antibodies as described in Section 2. (C) Effect of LY294002 on DEP-induced Akt activation. JB6 P^+ cells were pretreated with LY294002 (0 or 10 μM) for 30 min and exposed to DEP (10 μg/ml). Phosphorylation of Akt was determined as described above. (D) Phosphorylation of Akt in $JB6^{DNp85}$ cells. The JB6 P^+ cells stably expressing dominant-negative p85 subunit of PI3K ($JB6^{DNp85}$) were exposed to DEP (10 μg/ml), and phosphorylation of Akt was determined as described above. The experiments were replicated three times.

pathway. Blockage of PI3K activity by LY294002 eliminated DEP-mediated phosphorylation of p85/p70 S6 kinases, and partially inhibited DEP-induced GSK-3β phosphorylation on Ser-9 (Fig. 5).

The effect of DEP on MAPKs was also examined. DEP modestly activated SAPK/JNK pathway. As shown in Fig. 6, DEP induced a transient activation of MKK4, an up-stream signaling component of SAPK/JNK pathway,

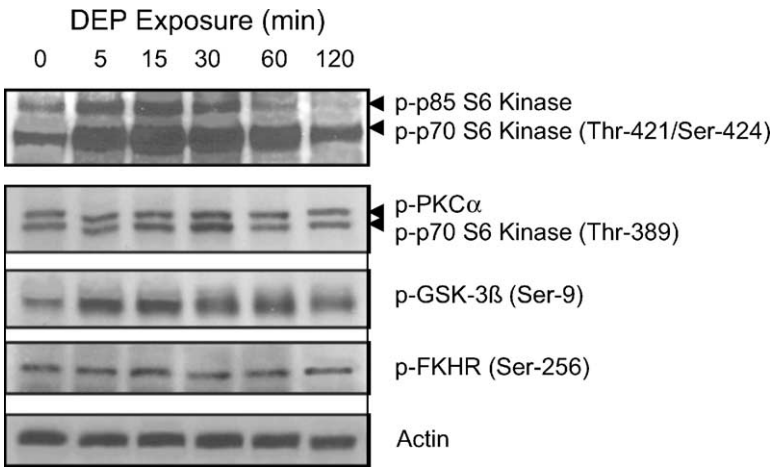

Fig. 4. Effect of DEP on Akt-regulated signaling in JB6 P^+ cells. Phosphorylation of p70 S6 kinase (Thr-389 and Thr-421/Ser-424), GSK-3β (Ser-9) and FKHR (Ser-256) was determined with immunoblots using phospho-specific antibodies. The same blots were stripped and probed with an anti-actin antibody. The experiments were replicated three times.

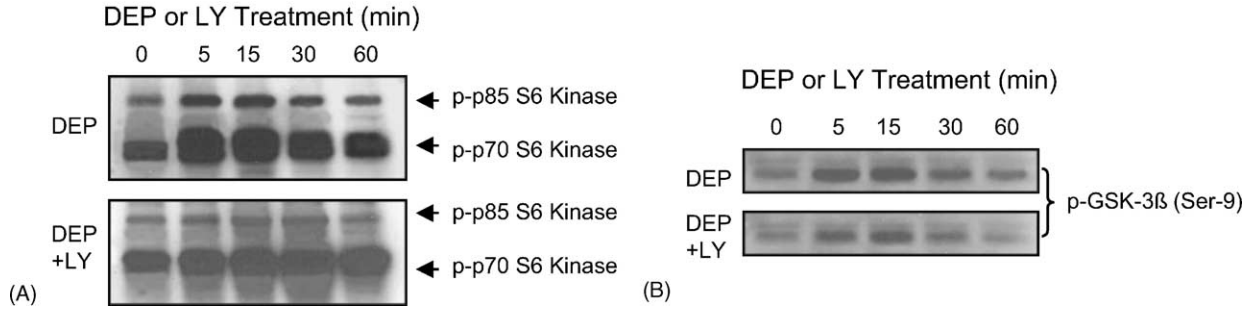

Fig. 5. Effect of LY294002 on DEP-induced activation of p70 S6 kinase and GSK-3β. JB6 P^+ cells were pretreated with LY294002 (0 or 10 μM) for 30 min and exposed to DEP (10 μg). Phosphorylation of p70 S6 kinase (Thr-421/Ser-424) (A) and GSK-3β (Ser-9) (B) was determined as described above. The experiments were replicated three times.

and enhanced phosphorylation of SAPK/JNKs. DEP also modestly promoted the phosphorylation of two downstream effectors of SAPK/JNKs, the c-Jun and ATF-2. On the other hand, DEP did not affect the phosphorylation of either p38 MAPK or ERKs.

3.4. DEP-stimulated NF-κB activity through PI3K/Akt signaling pathway

As shown in Fig. 7, treatment with PI3K inhibitor LY294002 completely eliminated DEP-stimulated NF-κB activity. A similar blocking effect was observed when another PI3K inhibitor wortmannin was applied (data not shown). TDZD-8 is a specific GSK-3β inhibitor [24]. D-JNKI is a specific JNK inhibitor that we have previously shown that it eliminated JNK activity [25]. Both TDZD-8 and D-JNKI had little effect on DEP-mediated NF-κB activation. The effect of other MAPK inhibitors on DEP-stimulated NF-κB activity was also examined. As expected, neither SB202190 (inhibitor for p38 MAPK) nor PD98059 (inhibitor for MEK1) altered DEP-regulated NF-κB activity (data not shown).

4. Discussion

Diesel exhaust particles have been shown to induce intensive inflammatory reactions and enhance allergic

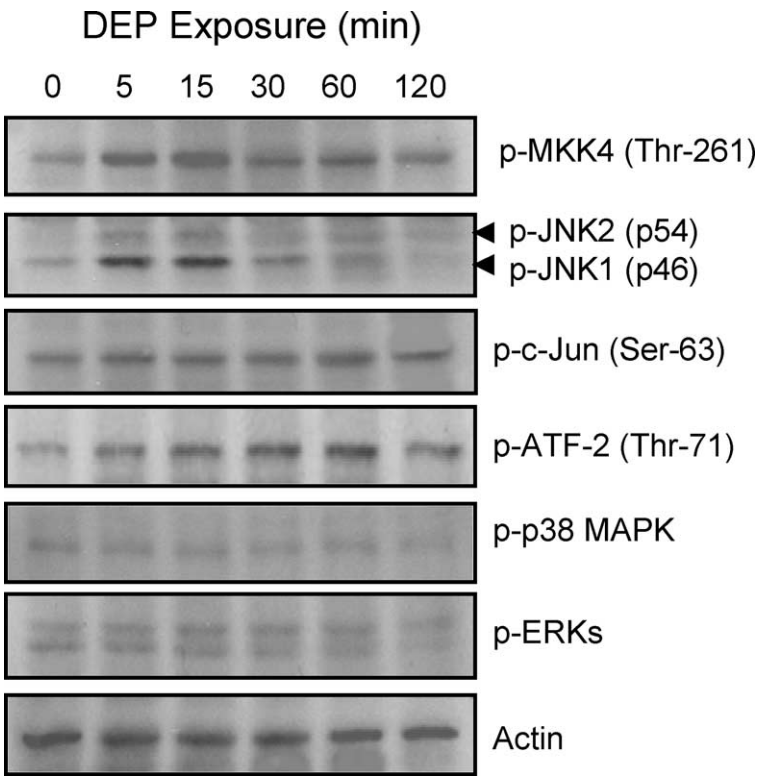

Fig. 6. Effect of DEP on MAPK signaling pathway in JB6 P^+ cells. Phosphorylation of MKK4, JNKs, c-Jun, AFT-2, ERKs and p38 MAPK was investigated with immunoblots using phospho-specific antibodies. The same blots were stripped and probed with an anti-actin antibody. The experiments were replicated three times.

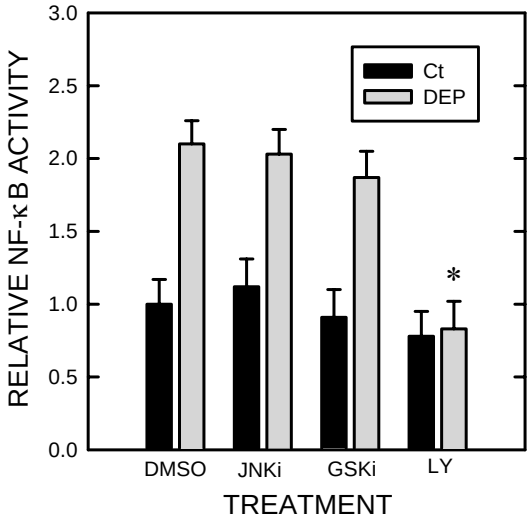

Fig. 7. Effect of inhibitors for PI3K, JNKs and GSK-3β on DEP-induced NF-κB activation in JB6 P^+ cells. JB6 P^+ cells were pretreated with 10 μM LY294002 (LY, PI3K inhibitor), 1 μM D-JNKI (JNKi, JNK inhibitor) and 10 μM TDZD-8 (GSKi, GSK-3β inhibitor) for 30 min and exposed to DEP (10 μg/ml, 12 h). Control cultures were treated with DMSO. Relative NF-κB activity was determined as described above. Each data point (±S.E.M.; bars) is the mean of four replicates. Asterisk (*) denotes a statistically significant difference from controls (DMSO) ($P < 0.05$).

responses [3,4,11,26]. These responses are largely mediated by DEP-induced production of pro-inflammatory cytokines [10], IgE [7] and adhesion molecules [9]. The production of these inflammatory and allergic mediators is regulated by the activity of transcription factors. NF-κB is an important transcription factor that regulates the gene transcription of a wide array of pro-inflammatory cytokines (interleukin-1β, -2, -6, -8, -12, TNF-α, GM-CSF, macrophage inflammatory protein-1α, monocyte chemotactic protein, GRO-α, -β, -γ, eotaxin and RANTES), adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin) and immune receptors (interleukin-2 receptor, T-cell receptor β chain and platelet-activating factor receptor) [4,10,27–29].

The epidermis is a rich source of pro-inflammatory cytokines and other allergic mediators [15]. Like airway epithelial cells, the epidermal cells are among the first cell populations that are exposed to chemical pollutants including DEP. We investigate the effect of DEP on the transactivation of NF-κB and AP-1 in a mouse epidermal cell line (JB6 P^+ cells) stably expressing NF-κB or AP-1 luciferase reporter constructs. These cells have been previously used as a valuable model to investigate NF-κB and AP-1 activation in epidermal cells [19,20,30]. We demonstrate here that exposure to DEP, at the non-cytotoxic concentrations (5–20 μg/ml), activates NF-κB but not AP-1. This concentration range is relevant to human exposure to DEP. The air concentration of DEP nationwide is around 2–5 $\mu g/m^3$, but in certain areas, the air level of DEP can be considerably higher. In the Los Angles Basin, one estimate has placed the rate of DEP intake by human at 300 μg every 1–3 days [31]. Similar to our finding, several studies demonstrate that DEP can activate NF-κB but not AP-1 in human bronchial epithelial cells [4,11,32]. Thus, NF-κB appears a target of DEP action and may mediate DEP-stimulated cytokine transcription. DEP are composed of a carbonaceous core with adsorbed organic compounds, sulfates and traces of heavy metals. It is currently unknown which component of DEP is responsible for NF-κB activation in epidermal cells. It is possible that the activation is mainly mediated by adsorbed organic compounds because these compounds could represent up to 60% of the mass of the particles and are involved in the activation of MAPKs and NF-κB in human airway epithelial cells [32].

The activity of NF-κB is regulated by multiple signaling pathways. Among these pathways, phosphatidylinositol 3-kinase (PI3K)/Akt- and mitogen-activated protein kinase (MAPK)-mediated signaling pathways play a critical role [27,30,33–35]. Akt is a substrate of PI3K; the activated PI3K phosphorylates Akt on Thr-308 and Ser-473, which results in full activation of Akt [36]. Our results indicate that PI3K/Akt signaling pathway is necessary for DEP-induced NF-κB activation because DEP activate Akt and blockage of PI3K by specific inhibitors (LY294002 and wortmannin) abolishes DEP-mediated NF-κB activation. Similarly, a recent study demonstrates that blockage of PI3K by LY294002 eliminates epidermal growth factor-induced NF-κB activation and subsequent IL-8 production in human head and neck squamous cell carcinoma lines [37]. The MAPKs, namely, the SAPK/JNKs, p38 MAPK and ERKs, are known to mediate NF-κB activity in response to various stimuli [30,33,38]; however, they are minimally involved in DEP-induced NF-κB activation in JB6 P^+ mouse epidermal cells. Although SAPK/JNK pathway is modestly and transiently activated by DEP, the extent of activation apparently is not sufficient to promote either AP-1 or NF-κB activity. DEP are ineffective in the activation of ERKs and p38 MAPK in JB6 P^+ mouse epidermal cells. However, other studies demonstrate that DEP are capable to activate ERKs and p38 MAPK in human airway epithelial cells [26,32,39]. Therefore, the effect of DEP is apparently cell type specific.

PI3K is an important signaling molecule which consists of a heterodimer of a 110-kDa (p110) catalytic subunit and an 85-kDa (p85) regulatory subunit. Upon activation, which is usually triggered by the association with tyrosine kinases or G-protein-coupled receptors, it phosphorylates phosphatidylinositol (PtdIns) and generated a phosphorylated derivative (phosphoinositide), such as PtnIns3P, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 [23]. Phosphoinositides interact with the pleckstrin homology (PH) motif and subsequently activate PH domain containing proteins. Akt is among the first protein known to contain a PH domain. The PH domain of Akt specifically binds PI3K lipid products, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. Resting cells contain substantial levels of PtnIns3P, but hardly any PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. Upon activation

of PI3K, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 are synthesized on the cytoplasmic membrane and Akt interacts through its PH domain with these lipids. This induces (1) the translocation of Akt to the plasma membrane and (2) a conformational change which exposes Thr-308 (kinase domain) and Ser-473 (C-terminal regulatory domain) phosphorylation sites at Akt. The phosphorylation of Akt was then catalyzed by PDK1 on the cytoplasmic membrane [23]. Phosphorylation of both residues is essential for maximal activation of Akt in response to PI3K activation. The phosphorylation of Akt can be independent of PI3K [23]. Our result indicates that DEP stimulate phosphorylation of Akt on both Thr-308 and Ser-473 in a PI3K-dependent manner. On the other hand, DEP do not affect either the expression or the phosphorylation of PTEN. PTEN antagonizes the action of PI3K activity by dephosphorylating PtdIns(3,4,5)P_3 [40]. Thus, DEP-induced Akt activation is less likely mediated by inhibition of PTEN.

Since Akt is activated by DEP, we further investigate the effect of DEP on three major down-stream effectors of Akt, namely, glycogen synthase kinase-3β (GSK-3β), p70 S6 kinase (p70S6K) and forkhead transcription factor (FKHR). The mitogen-stimulated protein kinase p70S6K/p85S6K is a Ser/Thr kinase that plays an essential role in cell proliferation and growth. p70S6K phsophorylates the 40S ribosomal protein S6 and is involved in translational control of 5′-oligopyrimidine tract mRNAs [41,42]. The p85S6K, an isoform of p70S6K, is derived from the same gene and is identical to p70S6K except for 23 extra residues at the N-terminus that encode a nuclear localizing signal [41]. The activation of p70S6K can be PI3K/Akt-dependent or independent [43]. PI3K/Akt-dependent activation is mediated by mammalian target of rapamycin (mTOR). DEP apparently promote phosphorylation of p70S6K/p85S6K in a PI3K/Akt-dependent manner; blockage of PI3K eliminates DEP-induced phosphorylation of p70S6K/p85S6K.

GSK-3β is named for its ability to phosphorylate, and thereby inactivate glycogen synthase, a key regulatory process in the synthesis of glycogen. Recent evidence indicates that GSK-3β is a critical figure in many cellular signaling pathways that regulates cell proliferation, survival and transformation [44]. GSK-3β activity is regulated by serine (inhibitory) and tyrosine (stimulatory) phosphorylation. DEP stimulate GSK-3β phosphorylation on Ser-9, and this stimulation is partially inhibited by LY294002, suggesting that PI3K/Akt pathway only partially contributes to the activation. Serine phosphorylation of GSK-3β can be regulated by multiple kinases. These include Akt, PKC, p90Rsk and p70S6K [44]. Besides Akt, it is currently unknown what other kinase(s) is(are) involved in DEP-stimulated GSK-3β phosphorylation. Dependent on cell types, GSK-3β could either positively or negatively regulate NF-κB activation [29,,44]. Our results indicate that GSK-3β does not involve DEP-induced NF-κB activation in JB6 P^+ epidermal cells.

In summary, this is the first report demonstrating that DEP can modulate NF-κB activity in epidermal cells. It supports that the hypothesis that the activity of transcription factors is the target of DEP action. Furthermore, PI3K/Akt signaling pathway is necessary for DEP-induced activation.

Acknowledgments

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