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IL-6 Augmented Motility of Airway Epithelial Cell BEAS-2B via Akt/GSK-3β Signaling Pathway

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ABSTRACT

Cell migration plays a pivotal role in airway repair and remodeling involved in respiratory diseases such as asthma. Interleukin-6 (IL-6) and fascin-1 are involved in cell migration upon stimulation; however, the roles of IL-6 and fascin-1 in migration of airway epithelial cell remain sketchy. The present study was aimed to investigate influence of IL-6 on cell motility with emphasis on the association with fascin-1. Wound healing assay and transmigration assay were performed to examine effect of IL-6 on migration and invasiveness of human bronchial epithelial cell BEAS-2B. Level of mRNA expression was determined by RT-PCR and quantitative real-time RT-PCR (Q-PCR). Involvement of kinase and transcription factor signaling in IL-6-induced cell migration was investigated using immunoblot and specific inhibitors. IL-6 significantly augmented cell migration and invasiveness in parallel with elevated fascin-1 expression. Further investigation showed that IL-6 dose-dependently upregulated fascin-1 expression in both mRNA and protein levels. We showed that IL-6 activated Akt and inhibited glycogen synthase kinase-3β (GSK-3β), highly associating with fascin-1 mRNA expression. Additionally, IL-6-induced migration was significantly diminished by phosphatidyl inositol 3-phosphate kinase (PI3K) inhibitor (wortamannin) and β-catenin inhibitor FH535. Moreover, LiCl and SB216763, inhibitors of GSK-3β augmented cell migration as well as fascin-1 mRNA expression. Conclusively, these findings reveal that IL-6-induced migration of BEAS-2B cell may be attributed to activation of Akt, inhibition of GSK-3β, and the associated increase of β-catenin and fascin-1 expression, indicating an important role of Akt/GSK-3β signaling and β-catenin/fascin-1 in IL-6 associated airway remodeling. J. Cell. Biochem. 113: 3567–3575, 2012.

KEY WORDS: IL-6; CELL MIGRATION; FASCIN-1; AKT; GSK-3β

Airway remodeling is a pathophysiolgical process in response to airway injury and associated with poor outcomes of lung disorders such as asthma and chronic obstructive pulmonary diseases (COPD). It is characterized by airway wall thickening, smooth muscle hypertrophy, and mucus hypersecretion. During initial stages of remodeling process, migration of airway epithelial cells toward the wound edge is stimulated leading to formation of temporary barrier [Davies, 2009], and the response is not accompanied with cell proliferation [Erjefalt et al., 1995]. In addition, down-regulation of tight junction proteins and increased expressions of matrix metalloproteinases (MMPs) and collagen have been reported to be present in the damaged airway epithelium [Vermeer et al., 2009; Xiao et al., 2011].

Mounting evidences have shown that airway epithelial cells play critical roles not only in physical resistance against pathogen invasion and allergens, but also in development of respiratory diseases such as asthma through activation of immune responses [Martin and Frevert, 2005; Holgate, 2008]. A variety of pro-inflammatory cytokines and chemokines are produced by airway epithelial cells in response to exposure to pathogens and allergens,

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including interleukin (IL)-6, IL-8, granulocyte macrophage-colony stimulating factor (GM-CSF), and monocyte chemotactic protein (MCP)-1 [Bhat et al., 2003; Osterlund et al., 2009; Roschmann et al., 2011]. Among aforementioned biomediators, IL-6, a multifunctional cytokine, is produced by various cell types, including B cells, macrophages, dendritic cells and epithelial cells [King et al., 1998]. IL-6 has received significant attention for its involvement in a variety of crucial biological functions and pathogenesis. It has been reported that IL-6 contributes to airway remodeling process [Pedroza et al., 2011] and enhances migration of various cells including cerebral endothelial cells [Yao et al., 2006], keratinocyte [Gallucci et al., 2004], corneal epithelium cells [Nishida et al., 1992] and biliary epithelial cell [Jiang et al., 2010]. These findings indicate that IL-6 plays a pivotal role in regulation of cell migration associated with remodeling.

Previous studies have shown that expression of fascin-1, a 55-kDa actin-bundling protein, is highly associated with epithelial cell migration in a stimulatory fashion [Yamashiro et al., 1998; Jawhari et al., 2003]. Regulation of fascin-1 expression is attributed to presence of cytokines including IL-6, tumor necrosis factor-alpha (TNF-α) and insulin-like growth factor-1 (IGF-1), leading to augmented invasiveness of human epithelial carcinomas [Guvakova et al., 2002; Onodera et al., 2009; Li et al., 2010]. However, the mechanisms underlying IL-6-mediated regulation of fascin-1 expression in airway epithelial cell are not completely understood.

In the present study, we aimed to investigate whether IL-6 promotes migration of airway epithelial cells and the underlying mechanisms with emphasis on fascin-1 involvement. Cell motility of transformed human bronchial epithelial cell BEAS-2B was analyzed by using wound healing assay and cell migration assay. mRNA expression levels were determined by RT-PCR and quantitative real-time RT-PCR (Q-PCR). Involvement of signaling pathways was investigated using immunoblotting and specific inhibitors.

MATERIALS AND METHODS

CELL CULTURE AND TREATMENTS

Immortalized normal human bronchial cell BEAS-2B, purchased from American Type Culture Collection, was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Invitrogen Life Technologies, Carlsbad, CA), 2 mM L-glutamine and 100 U/ml penicillin–streptomycin (Sigma–Aldrich, St. Louis, MO) at 37°C in a humidified atmosphere with 5% CO_2. Cells were grown on collagen/fibronectin-coated tissue-culture plates until 90% confluence and passaged by trypsinization.

For treatment, cells were seeded in 6-cm sterile Petri dish at 3×10^5 cells/ml, cultured in the complete medium until 90% confluence, and then starved in serum-free DMEM for 16 h. The starved cells were incubated with IL-6 (R&D Systems, Minneapolis, MN) or pretreated with specific inhibitors, including LiCl and SB216763 (glycogen synthase kinase-3 beta [GSK-3β]), PD98059 (MEK), wortmannin (phosphatidylinositol 3-kinase [PI3K]), S31-201 [STAT3], KG-501 (Naphthol AS-E phosphate; cAMP response binding protein [CREB]) [Best et al., 2004], FH535 [Wnt/β-catenin] [Handeli and Simon, 2008], and JSH-23 (4-methyl-N1-(3-phenylpropyl)-benzene-1,2-diamine) [NF-κB] [Shin et al., 2004] (Sigma–Aldrich) at indicated conditions, and followed IL-6 (20 ng/ml) treatment.

CELL VIABILITY ASSAY

Cells viability was determined using MTT assay as described previously [Mosmann, 1983]. Briefly, cells with different treatments were incubated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma–Aldrich] in DMEM (0.5 mg/ml) for 4 h at 37°C. After removal of supernatant and addition of isopropanol, absorbance at 565 nm was measured using an ELISA reader. Cell viability was proportional to the absorbance at 565 nm.

WOUND HEALING ASSAY

Cells were seeded on 6-cm dishes at 1×10^6 cells/ml and cultured in DMEM supplemented with 10% FBS until 90% confluence, followed by starvation with serum-free DMEM for 16 h. The wound line was generated by sculpturing cell culture using a sterile 10 μl-pipette tip, and the cells were subsequently exposed to IL-6 or inhibitors in DMEM at indicated concentration for 24, 48, or 72 h. Images of resulting culture were obtained at 0, 24, 48, and 72 h using a light microscopy with digital camera.

TRANSMIGRATION ASSAY

The ability of cell transmigration was determined by using 24-well Transwell plates (pore size: 8 μm; Costar, Cambridge, MA) as previously described [Lin et al., 1996]. 5×10^4 of cells were seeded onto the upper chamber and IL-6 (20 ng/ml) in serum-free DMEM was placed in the lower chamber. After 24 h incubation, the cells were fixed with 100% methanol for 15 min, and then stained with 0.05% crystal violet (Sigma–Aldrich) in phosphate-buffered saline (PBS) for 15 min. The cells on the upper surface of the membrane were mechanically scraped, and the cells that had transmigrated to the lower surface of the membrane were counted. The average number of transmigrated cells from five randomly chosen observing fields on the lower surface of the membrane was counted. Three independent experiments were performed for statistical analysis.

IMMUNOBLOTTING

After, treatment, cells were washed with PBS and harvested and lysed in the lysis buffer [10 mM Tris–HCl, pH 7.5; containing 1% v/v Triton X-100, 150 mM NaCl, 0.5 mM EDTA, 1 mM phenylmethanesulfonylfluoride (PMSF), 1 mM NaF, 1 mM NaP_2O_7, and 10 μg/ml Aprotinin and Leupeptin (Sigma–Aldrich)]. Cell lysates were centrifuged and the supernatants were collected for SDS–PAGE analysis. Protein concentration of supernatant was determined using BCA protein assay kit (Pierce Biotechnology). The crude proteins (30 μg/lane) were subject to a 12.5% SDS–acrylamide gel, and then transferred onto nitrocellulose membrane (Millipore, Bedford, MA). The resulting membranes were blocked with 5% (w/v) skimmed milk and subsequently incubated with primary antibodies against phosphorylated ERK1/2 (p-ERK1/2), ERK1/2, phosphorylated AKT (pAKT), AKT (Cell Signaling Technology, Beverly, MA), fascin-1 (Santa Cruz) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Abcam, Cambridge, UK). After washing with PBS containing 0.1% (v/v) Tween-20, the incubated membranes were exposed to anti-IgG antibodies conjugated with peroxidase

(MoBioPlus, Taipei, Taiwan). The detection of antigen–antibody complex was performed using ECL reagent (Millipore, Bedford, MA) and luminescence image system (LAS-4000; Fujifilm, Tokyo, Japan).

RNA EXTRACTION, RT-PCR AND Q-PCR

Isolation of total RNA was performed by using RNeasy mini kit (Qiagen, Hilden, Germany) and treated with DNase I according to the manufacturer's instructions. First strand cDNA was synthesized from 1 μg total RNA by reverse transcription in a 20 μl reaction using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.) following the manufacturer's instruction. All PCR assays were performed in a 50 μl reaction mixture containing 2.5 μl cDNA and 200 nM of each primer using PCR master mix. The temperature cycle profile for the PCR reactions was 94°C for 5 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by 72°C for 7 min. Primers used for RT-PCR and Q-PCR were designed by using Primer Premier version 5.0 (Premier Biosoft, Palo Alto, CA) and listed below: fascin-1(F): 5′-ACC TGT CTG CCA ATC AGG AC-3′, fascin-1(R): 5′-CCC ATT CTT CTT GGA GGT CA-3′, GAPDH(F): 5′-ATG CCT CCT GCA CCA CCA-3′, GAPDH(R): 5′-CCA TCA CGC CAC AGT TTC C-3′. GAPDH primers were used as internal control. Agarose gel electrophoresis (2%) and direct DNA sequencing methods were utilized to confirm specificity of the PCR products. Q-PCR was performed using the ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). For mRNA quantitation, FastStart Universal SYBR Green Master (Roche Applied Science, Mannheim, Germany) was used for Taqman PCR. The threshold cycle numbers were calculated using the ΔΔCT relative value method and normalized to GAPDH. Q-PCR experiments were performed in duplicates for each sample. The correct size of the PCR products was confirmed by agarose gel electrophoresis.

IMMUNOFLUORESCENCE STAINING

Cells were seeded onto a 6-cm dish at a density of 1×10^6 cells/ml, and cultured in DMEM with 10% FBS until 90% confluent. After starvation in serum-free DMEM for 16 h, the cells were washed with PBS and incubated with IL-6 for 24 h. The cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min, and then treated with 0.5% (v/v) Triton X-100 in PBS for 5 min. The treated cells were incubated with 10% (w/v) bovine serum albumin in PBS for 1 h at 37°C, and then reacted with 1/100 diluted anti-fascin-1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 16 h. After washing with PBS, the reacted cells were incubated with 1/200 diluted secondary antibody conjugated with DyLight 649 (BioLegend, San Diego, CA) for 2 h. Signals were detected by fluorescence microscopy and the nucleuses were indicated by DAPI staining.

STATISTICAL ANALYSIS

Data were presented as means ± SD of three independent experiments. Statistical significance analysis was determined by using one-way ANOVA followed by Dunnett for multiple comparisons with the control. The differences were considered significant for $P < 0.05$.

RESULTS

IL-6 PROMOTED MOTILITY OF BEAS-2B CELL ASSOCIATING WITH FASCIN-1 EXPRESSION

Before examining whether IL-6 affecting cell motility, we initially determined whether IL-6 has effects on airway epithelial cell viability. As shown in Supplementary Figure 1, cell viability of human bronchial epithelial cell BEAS-2B was unaffected by incubation with different concentrations of IL-6 for 24 or 48 h compared to the mock controls. Therefore, the effects of IL-6 on migration of BEAS-2B cells were then determined by using wound healing assay. As shown in Figure 1A, IL-6 (0–20 ng/ml) promoted migration of BEAS-2B cell in a dose-dependent manner. It is evident that manipulation of the actin cytoskeleton plays a critical role in enhancement of cell motility. Accordingly, the involvement of

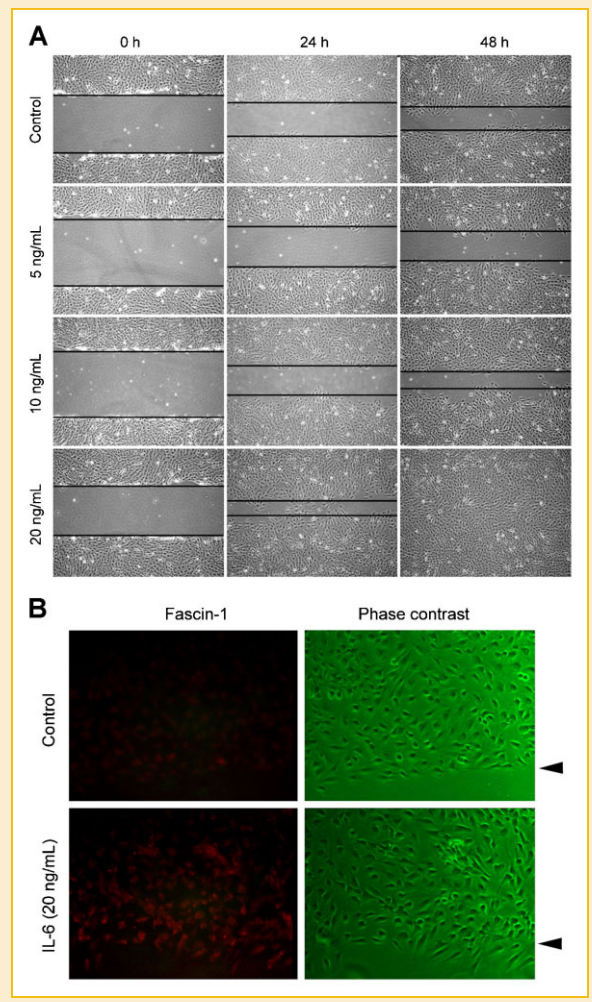

Fig. 1. Effects of IL-6 on cell migration and fascin-1 expression of human bronchial epithelial cell BEAS-2B. A: Cells were treated with IL-6 at indicated concentrations for 24 or 48 h, and then the cell migration was demonstared by using wound healing assay as described in Materials and Methods Section. B: Cells were treated with IL-6 (20 ng/ml) for 24 h, and then the fascin-1 expression was monitored by immunofluorescence assay (red). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

actin-bundling protein fascin-1 in cell migration in response to IL-6 treatment was investigated. Our results showed that IL-6 elevated protein level of fascin-1 in BEAS-2B cells, particularly in the cells near the trench (Fig. 1B).

In addition to examining cell migration on surface, effects of IL-6 on cell transmigration across membrane of BEAS-2B were also demonstrated by using transmigration assay. Our results showed that IL-6 significantly increased number of BEAS-2B cells transmigrated across membrane in a dose-dependent manner (Fig. 2A,B; $P < 0.05$ as compared to control), and the transmigrated cells upon IL-6 stimulus (20 ng/ml) expressed a higher level of fascin-1 as compared to control (Fig. 2C). Taken together, these findings revealed that IL-6 promoted both the migration and the transmigration activity of BEAS-2B cells associating with increased level of fascin-1.

IL-6 UPREGULATED mRNA EXPRESSION AND PROTEIN LEVEL OF FASCIN-1 IN BEAS-2B CELL

Since level of fascin-1 is associated with IL-6 induced cell migration and transmigration, whether IL-6 regulates mRNA expression and alters cellular level of fascin-1 is investigated. As shown in Figure 3A, RT-PCR analysis showed that IL-6 treatment increased mRNA expression of fascin-1 in BEAS-2B cell. Further quantitative analysis by Q-PCR revealed that IL-6 significantly elevated mRNA expression of fascin-1 in a dose-dependent manner ($P < 0.05$, Fig. 3B). In addition to mRNA expression, the cellular level of fascin-1 was also determined by immunofluorescence assay. As shown in Figure 3C, IL-6 dose-dependently increased level of cellular fascin-1 in BEAS-2B cells. These findings supported elevation of expression of fascin-1 in BEAS-2B in response to presence of IL-6.

IL-6 INDUCED FACSIN-1 EXPRESSION IN BEAS-2B CELL VIA MEK/ERK AND PI3K/AKT SIGNALING PATHWAYS

IL-6 has been documented to regulate its downstream gene expression through several signaling pathways including JAK/STAT, MEK/ERK, and PI3K/AKT [Neurath and Finotto, 2011]. Our results showed that IL-6 treatment (20 ng/ml) induced phosphorylation of AKT and ERK1/2 in BEAS-2B cell (Fig. 4A). Using specific inhibitors for JAK/STAT, MEK/ERK, and PI3K/AKT pathways, we showed that, inhibition of MEK and PI3K signaling by PD98059 and wortamannin significantly diminished the mRNA level of fascin-1 induced by IL-6 in BEAS-2B cell (Fig. 4B). By contrast, mRNA level of fascin-1 in BEAS-2B cell was insignificantly affected through inhibition of STAT3 signaling by S31-201. In addition, the similar findings were obtained at quantitation of mRNA expression using Q-PCR in the same experimental setting (Fig. 4C). In parallel, the effects of the inhibitors on protein level of fascin-1 in BEAS-2B cells stimulated with IL-6 were analyzed by immunoblotting, and the results were consistent with that on mRNA level (Supplementary Fig. 2A). Moreover, inhibitory effects of S31-201 on phosphorylation of STAT3 in BEAS-2B cells upon IL-6 treatment were also demonstrated (Supplementary Fig. 2B). Together, these findings revealed that MEK/ERK and PI3K/AKT signaling play important roles in upregulated expression of fascin-1 in BEAS-2B cell induced by IL-6.

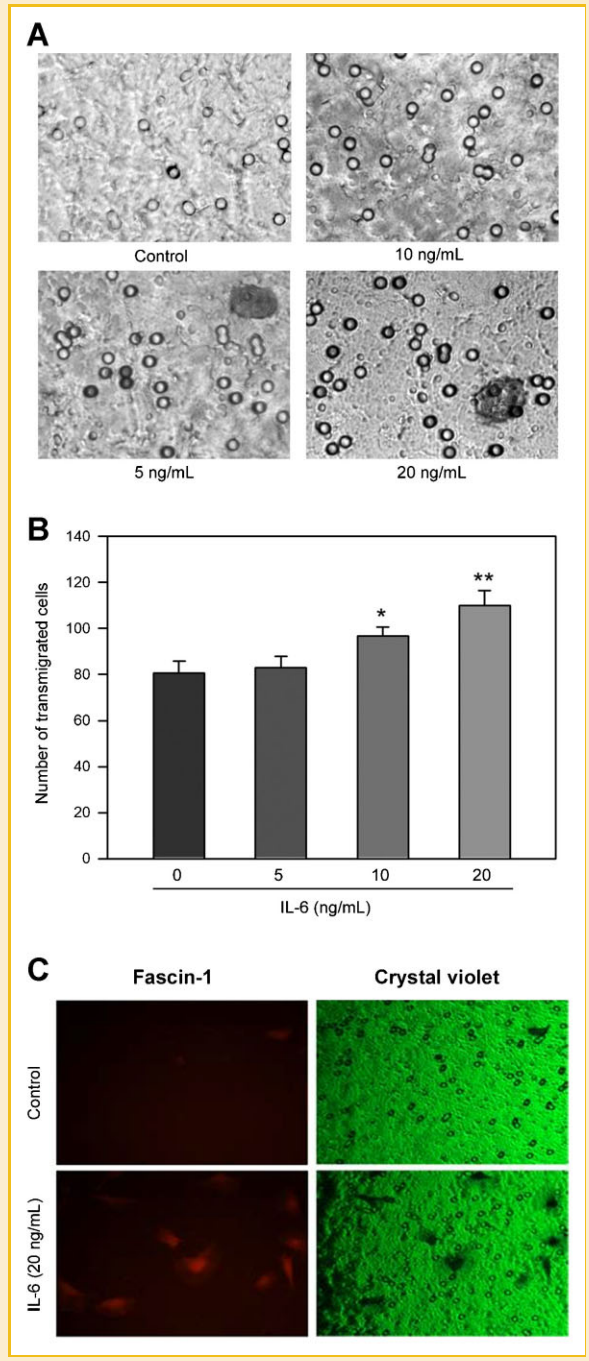

Fig. 2. IL-6 promoted cell transmigration and fascin-1 expression of human bronchial epithelial cell BEAS-2B. A: Cells were treated with IL-6 at indicated concentrations for 24 h, and then the cells penetrated through membrane were monitored by crystal violet staining for cell counting. B: The number of the cells penetrated through membrane were obtained from five randem observation fields. * and **, $P < 0.05$ and $P < 0.005$ as compared to the control. C: Cells were treated with IL-6 (20 ng/ml) for 24 h, and then the cells penetrated through membrane were monitored by crystal violet staining and immunofluorescence staining for determing Fascin-1 expression (red). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

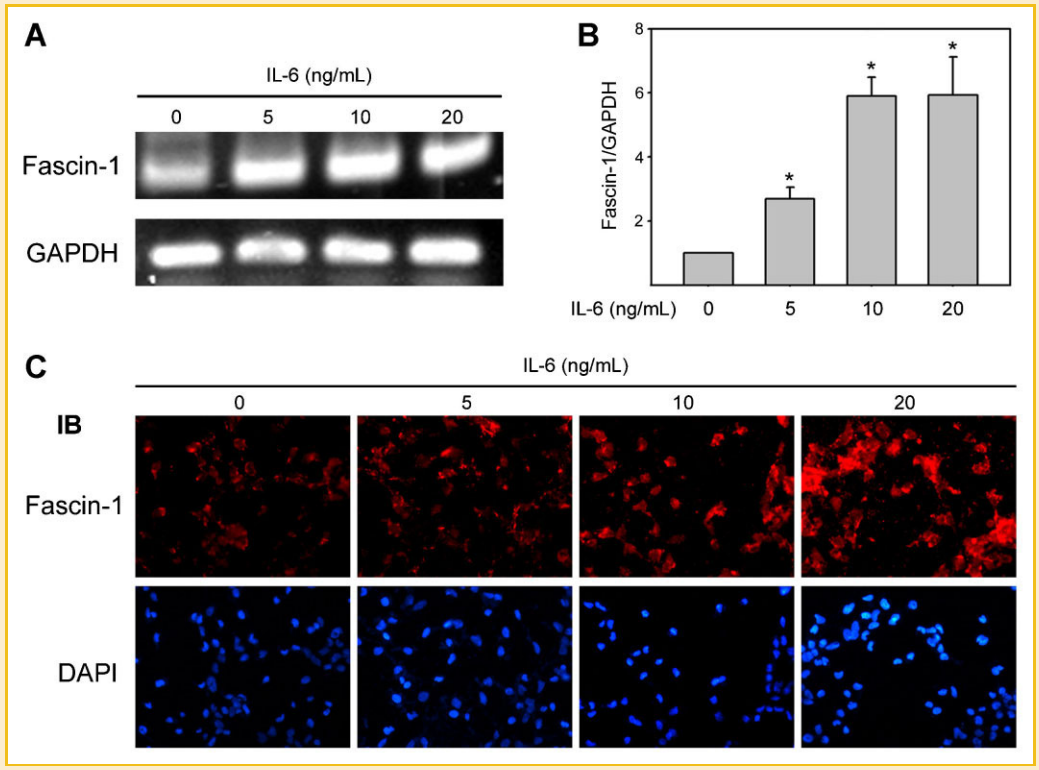

Fig. 3. IL-6 upregualted fascin-1 expression in human bronchial epithelial cell BEAS-2B. Cells were treated with IL-6 at indicated concentration for 24 h, and then lyzed for extraction of mRNA analysis or performed immunofluorescence assay. A: mRNA expression was analyzed by RT-PCR. B: mRNA level was quantitated by using Q-PCR. C: Protein on cell surface was analyzed by immunofluorescence staining (red). Nucluses were observed by DAPI staining (blue). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

INHIBITORY PHOSPHORYLATION OF GSK-3β ASSOCIATED WITH FACSIN-1 EXPRESSION OF BEAS-2B CELL INDUCED BY IL-6

GSK-3β is a downstream substrate of AKT kinase and can be inactivated through phosphorylation of serine-9 (pS9) residue. Hence, we investigated whether the inhibitory phosphorylation of GSK-3β associates with fascin-1 expression in IL-6 stimulated BEAS-2B cell. As shown in Figure 5A, IL-6 treatment (20 ng/ml) obviously increased level of serine-9 phosphorylation of GSK-3β (pS9-GSK-3β). In parallel, the inhibitory phosphorylation of GSK-3β triggered by the inhibitor LiCl (5, 10, and 20 mM) dose-dependently elevated the mRNA expression of fascin-1 up to 18.3 ± 3.3-fold as compared to control in BEAS-2B cell by using Q-PCR (Fig. 5B). In addition to LiCl, SB216763 (10 μM), a specific GSK-3β inhibitor, also increased mRNA expression of fascin-1 up to 15.9 ± 2.1-fold as compared to control in BEAS-2B cells by using Q-PCR (Fig. 5C). Thus, these results showed that IL-6 triggered phosphorylation of serine-9 at GSK-3β, attributing to promotion of fascin-1 mRNA expression in BEAS-2B cells.

IL-6 AUGMENTED MOTILITY OF BEAS-2B CELL VIA PI3K/AKT/GSK-3β AND β-CATENIN SIGNALING PATHWAY

Since the inhibition of GSK-3β leads to the upregulation of fascin-1, the signaling pathways involved in cell migration in response to IL-6, including inhibition of GSK-3β and the transcription factors linking to mRNA expression of fascin-1 [Snyder et al., 2011], was further elucidated by wound healing assay and transmigration assay upon specific inhibitors. As shown in Figure 6A, IL-6 treatment enhanced the migration of BEAS-2B cell as compared to control, and the promoted migration was attenuated by pretreatment of PI3K inhibitor wortamannin, whereas MEK inhibitor PD98059 or STAT3 inhibitor S31-201 unaffected the IL-6-induced motility. In addition, both wortmannin combining with LiCl or LiCl only increased the migration of BEAS-2B cells.

For the inhibition of GSK-3β associating with the increased mRNA expression of fascin-1; therefore, roles of transcription factors, CREB, β-catenin and NF-κB that are activated by GSK-3β and involved in mRNA expression of fascin-1 were further investigated. As shown in Figure 6B,C, inhibition of β-catenin significantly diminished the IL-6 induced protein level and mRNA expression of fascin-1 ($P < 0.05$), whereas inhibition of CREB and NK-κB failed to do so. In addition, inhibition of β-catenin led to a marked attenuation in IL-6-enhanced cell motility ($P < 0.05$ as compared to IL-6 treatment; Fig. 6D) Taken together, these findings showed that PI3K/AKT/GSK-3β signaling and β-catenin activation play pivotal roles in promoted cell motility of BEAS-2B by IL-6.

DISCUSSION

Level of IL-6 in circulation is very low or undetectable in health people whereas patients with asthma exhibit a significant increase in

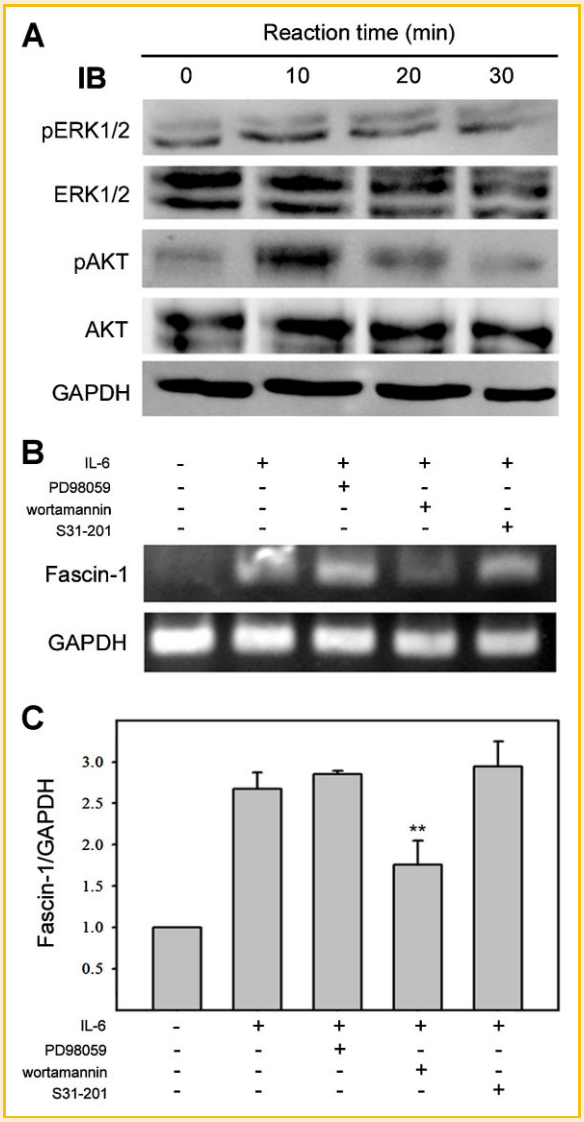

Fig. 4. Involvement of AKT and ERK1/2 activation in IL-6 induced fascin-1 expression in human bronchial epithelial cell BEAS-2B. A: Cells were treated with IL-6 (20 ng/ml) for 10, 20, or 30 min, and then lyzed for immunodetection using specific antibodies against the indicated targets. B: Cells were pretreated with indicated inhibitors for 1 h and then treated with IL-6 (20 ng/ml) for 24 h. The treated cells were lyzed for mRNA extraction, and then the mRNA was analyzed by RT-PCR (B) or quantitated by Q-PCR (C). **, $P < 0.005$ as compared to the control.

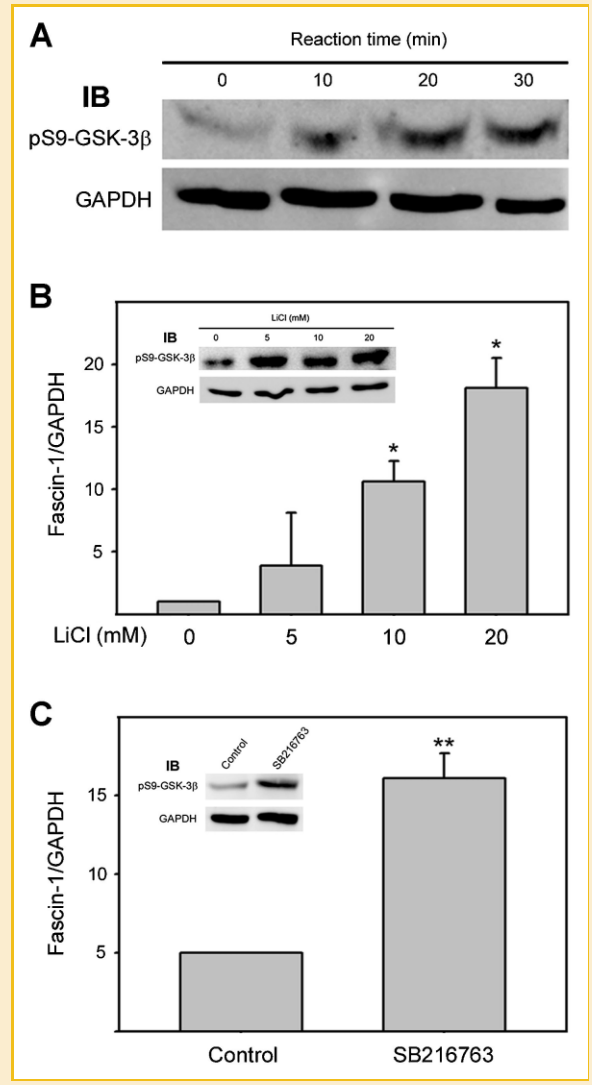

Fig. 5. Involvement of inhibitory phosphorylation of GSK-3β in fascin-1 expression in human bronchial epithelial cell BEAS-2B. A: Cells were treated with IL-6 (20 ng/ml) for 10, 20, or 30 min, and then lyzed for immunodetection of pS9-GSK-3β. Cells were treated with LiCl at indicated concentration (B) or SB216763 at 10 μM (C) for 4 h and then lyzed for mRNA and crude protein extraction. The extracted mRNA was quantitated by Q-PCR and the crude proteins were used for immunodetectin of pS9-GSK-3β. * and **, $P < 0.05$ and $P < 0.005$ as compared to the control.

IL-6 production, suggesting involvement of IL-6 in development and pathogenesis of asthma [Broide et al., 1992; Yokoyama et al., 1995]. However, the roles of excessive IL-6 level on airway epithelium remodeling are not completely understood. In the present study, we demonstrated that exposure of human bronchial cell BEAS-2B to IL-6 led to a significant enhancement in the motility, suggesting that increase of IL-6 in asthmatic patients may initiate the airway remodeling and consequently exacerbate the injury of respiratory epithelium.

IL-6 signaling pathway is known to be involved in various biological functions. The IL-6 receptor is a heterotrimeric membrane-associated protein, consisting of α-subunit (gp80) and β-subunit (gp130). Binding of IL-6 to gp80 induces homodimerization of gp130, and consequently activates the downstream pathways, including PI3K/AKT, MEK/ERK, and JAK2/STAT3 signaling [Neurath and Finotto, 2011]. Our results showed that IL-6 induced the activation of AKT, ERK1/2 and STAT3 in BEAS-2B cells. We further demonstrated that PI3K/AKT signaling is mainly associated with the upregulation of fascin-1 expression and the enhanced motility of BEAS-2B cells exposed to IL-6. In addition, we also provided evidences that inhibition of GSK-3β via serine-9 phosphorylation was involved in the migration of BEAS-2B cells in response to IL-6 treatment. Taken together, these findings indicate

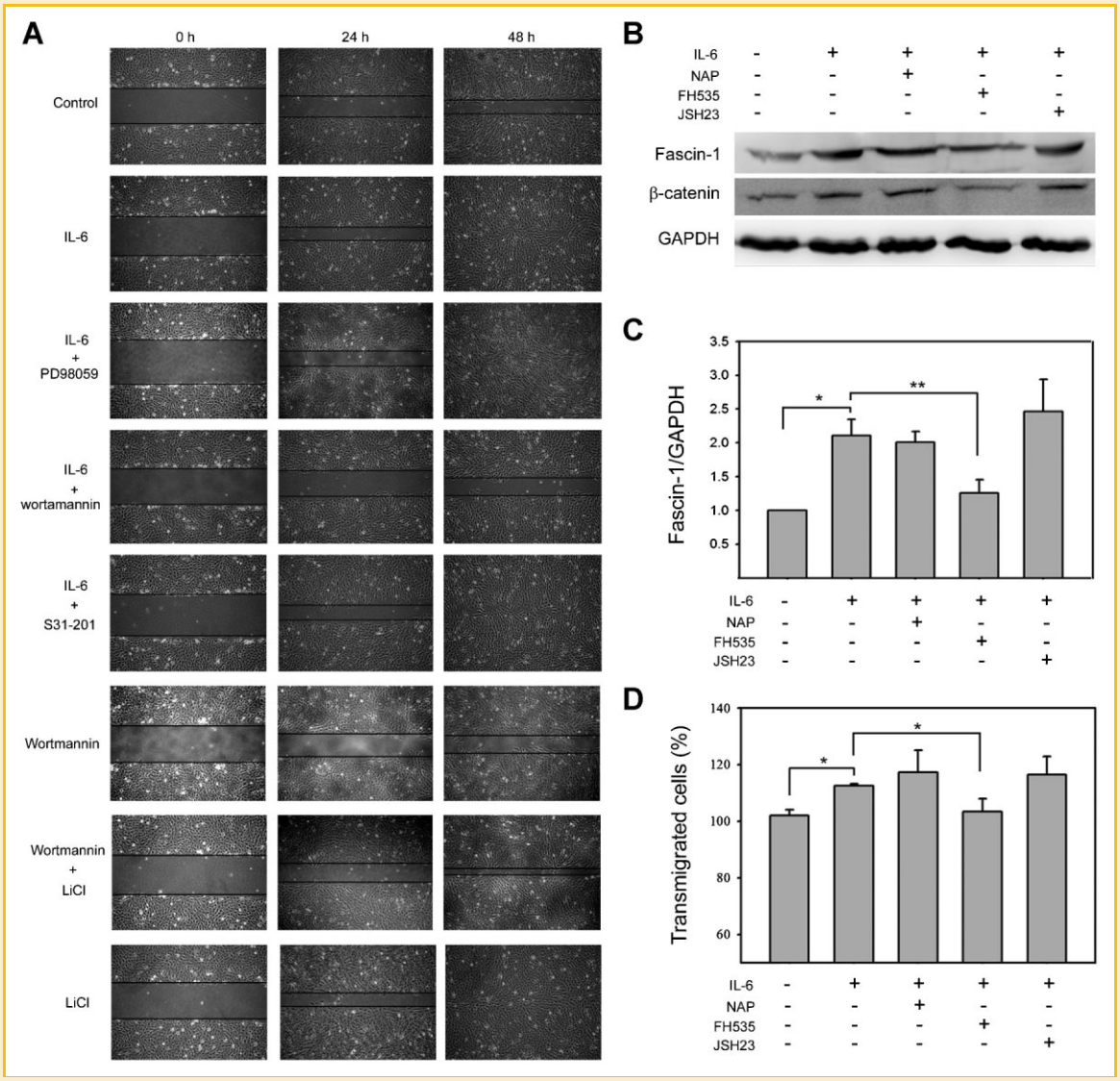

Fig. 6. Involvement of AKT, GSK-3β and β-catenin in cell motility and fascin-1 expression of human bronchial epithelial cell BEAS-2B in response to IL-6. Cells were pretreated with indicated inhibitors for 1 h and then treated with IL-6 (20 ng/ml) for 24 h. The migration of treated cells were determined by using wound healing assay (A), the crude proteins extracted from the treated cells were used for immunoblotting (B), the mRNA extracted from the treated cells was quantitated by Q-PCR (C), and the cell transmigrated across membrane was determined by transmigration assay (D). * and **, $P < 0.05$ and $P < 0.005$ as compared to the control.

that PI3K/AKT/GSK-3β signaling participates in the upregulation of fascin-1 and the promoted motility of airway epithelial cell in response to presence of IL-6.

Fascin is found to mainly localize at filopodia and membrane ruffles in Hela cells [Yamashiro-Matsumura and Matsumura, 1985], as well as in microspikes during spreading of a variety of cells including glioma cells [Lin et al., 1996] and myoblasts [Adams, 1995]. Previous study has shown that highly expression of Fascin in epithelial cell LLC-PK1 causes disorganization of cell-cell contacts and greatly increases cell migration activity up to 8–17 folds [Yamashiro et al., 1998]. Our results revealed that IL-6 significantly increased both mRNA expression of fascin-1 and cell motility of BEAS-2B cell. In parallel, inhibition of fascin-1 expression by wortmannin or FH535 resulted in the suppression in IL-6-induced cell migration in BEAS-2B cells. It is suggested that increased cell motility of BEAS-2B cells in presence of IL-6 is probably attributed to the upregulation of fascin-1 expression.

Recent studies have shown that elevated fascin-1 expression via STAT3, NF-κB, and β-catenin-TCF signaling plays a pivotal role in cell migration of various cancer cells [Vignjevic et al., 2007; Hashimoto et al., 2009; Kress et al., 2011; Snyder et al., 2011]. In the present study, our results showed that inhibition of β-catenin, but not STAT3 or NF-κB, diminished IL-6 induced fascin-1 expression and cell migration of BEAS-2B cells. Thus, we propose that β-catenin signaling may play a more prominent role in normal airway epithelial cells as comparing to metastatic or invasive cancer cells.

β-Catenin-TCF signaling has been reported to elevate fascin-1 level via binding to the fascin-1 promoter and the consequent

increase of fascin-1 mRNA expression in colon cancer [Vignjevic et al., 2007; Kim et al., 2010]. Our previous study indicates that AKT-induced inhibitory phosphorylation of GSK-3β enhances nuclear translocation of β-catenin in human lung alveolar carcinoma A549 [Wang et al., 2011]. In the present study, our results showed that inhibitory phosphorylation of GSK-3β promoted both mRNA expression and protein level of fascin-1 in BEAS-2B cells, as well as enhanced the cell motility of BEAS-2B cells. Thus, it is indicated that inhibition of GSK-3β play a pivotal role in IL-6 induced fascin-1 expression, which may attribute to the activation of β-catenin-TCF signaling.

In conclusion, the present study demonstrates that IL-6 significantly enhances cell motility of human bronchial epithelial cell BEAS-2B, which may attribute to upregulating fascin-1 expression through activation of PI3K/AKT, inhibitory phosphorylation of GSK-3β, and induction of β-catenin signaling. These findings suggest that GSK-3β/β-catenin represents a potential target to alleviate progression of chronic respiratory disease.

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