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Ginger Suppresses Phthalate Ester-Induced Airway Remodeling

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ABSTRACT: This study has two novel findings: it is not only the first to demonstrate inflammatory cytokines, which are produced by the bronchial epithelium after exposure to phthalate esters and contribute to airway remodeling by increasing human bronchial smooth muscle cells (BSMC) migration and proliferation, but it is also the first to reveal that ginger reverses phthalate ester-mediated airway remodeling. Human bronchial epithelial cell lines BEAS-2B and HBE135-E6E7 (HBE) were treated with butylbenzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), dibutyl phthalate (DBP), and diethyl phthalate (DEP), and the conditioned medium (CM) was harvested and then added to BSMC. Cultures of BSMC with BBP-, BEHP-, DBP-, and DEP-BEAS-2B-CM and DEP-HBE-CM increased BSMC proliferation and migration, which are major features in asthma remodeling. Exposure of BEAS-2B and HBE to DBP caused epithelial cells to produce inflammatory cytokines IL-8 and RANTES, which subsequently induced BSMC proliferation and migration. Depleting both IL-8 and RANTES completely reversed the effect of DBP-BEAS-2B-CM and DBP-HBE-CM-mediated BSMC proliferation and migration, suggesting this effect is a synergistic influence of IL-8 and RANTES. Moreover, [6]-shogaol, [6]-gingerol, [8]-gingerol, and [10]-gingerol, which are major bioactive compounds present in *Zingiber officinale*, suppress phthalate ester-mediated airway remodeling. This study suggests that ginger is capable of preventing phthalate ester-associated asthma.

KEYWORDS: *Zingiber officinale*, ginger, phthalate, asthma, airway remodeling

INTRODUCTION

Humans have significant exposures to phthalates, as these chemical plasticizers are ubiquitously present in flexible plastics.[1,2] Epidemiological data point to a possible correlation between phthalate exposure and asthma and airway diseases in children.[3−5] Experimental studies present support for an adjuvant effect in allergic sensitization by several phthalates.[5−7] However, the effect of phthalate esters used leading to increased airway remodeling is not well understood.

The major features of airway remodeling include loss of epithelial integrity, subepithelial fibrosis, goblet cell and submucosal gland enlargement, increased bronchial smooth muscle mass, and increased angiogenesis.[8] Human bronchial smooth muscle cells (BSMC) play a key role in the modulation of airway tone. In an asthmatic airway, BSMC not only increase secretory and proliferative ability but also migrate to the subepithelial area.[8,9] In addition, BSMC also release proinflammatory cytokines, which are responsible for the progression of asthma pathogenesis. The degree of change in bronchial smooth muscle mass has been directly correlated to asthma severity.[8,9]

Ginger (*Zingiber officinale*) is a well-known plant used in cooking worldwide and has long been reputed to have medicinal properties. It is a herbaceous, rhizomatous perennial plant widely distributed throughout tropical and subtropical regions[10] and is cultivated on a large scale in Nigeria, India, Bangladesh, Sri Lanka, Taiwan, and other East Asian countries.[10] Ginger has been used since antiquity for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases, and helminthiasis.[11,12] [6]-Shogaol, an active ingredient in ginger, has also been shown to exert anti-inflammatory and anticancer effects.[13,14]

In this study, we have not only evaluated the effect of phthalate esters in airway remodeling but also investigated ginger as a potential antidote in the fight against phthalate ester-induced asthma.

MATERIALS AND METHODS

Chemicals. [6]-Shogaol, [6]-gingerol, [8]-gingerol, and [10]-gingerol (Figure 1) were purchased from Sigma Chemical Co. (St. Louis, MO), dissolved in dimethyl sulfoxide (DMSO), and stored at −20 °C. Control cultures received the carrier solvent (0.1% DMSO). All other chemicals used were in the purest form available commercially.

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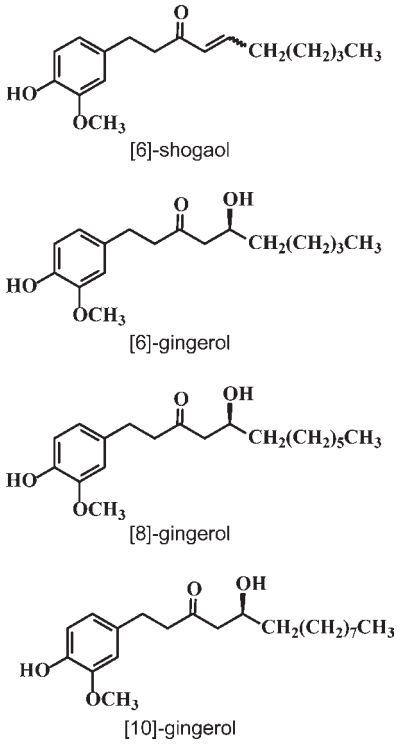

Figure 1. Chemical structures of [6]-gingerol, [8]-gingerol, [10]-gingerol, and [6]-shogaol.

Cell Culture and Condition Medium. Two human bronchial epithelial cell lines, BEAS-2B (CRL-9609) and HBE135-E6E7 (HBE, CRL-2741), obtained from ATCC, were used in our study. BEAS-2B was cultured in BEGM medium (Lonza, Walkersville, MD), and HBE135-E6E7 (HBE) was maintained in keratinocyte serum-free medium with 5 ng/mL human recombinant EGF and 0.05 mg/mL bovine pituitary extract (Invitrogen) supplemented with 0.005 mg/mL insulin and 500 ng/mL hydrocortisone. Primary human BSMC were obtained from Lonza and cultured in SmGM-2 smooth muscle medium (Lonza).

To obtain the various condition media, BEAS-2B and HBE cells (2×10^6 /100 mm dish) were treated with various concentrations of DBP for 6 h. After treatment, the medium was replaced and the supernatants were harvested after 24 h of incubation. Depletion of IL-8 and RANTES from various CMs was performed using anti-IL-8 or anti-RANTES antibodies (2 μg/mL, R&D System) and Sepharose A/G beads following regular immunoprecipitation techniques. Cytokine depletion was confirmed by IL-8 and RANTES ELISA assay kits.

Enzyme-Linked Immunosorbent Assay (ELISA). The levels of IL-8, RANTES, CXCL5, and IL-1β were determined by ELISA-based kits (R&D System Europe Ltd., Abingdon, U.K.). ELISAs were performed according to the manufacturer's instructions.

Cell Proliferation. BSMC (4×10^3/well) were plated in 96-well culture plates. After 24 h of incubation, the cells were treated with vehicle control-CM or various condition media for 72 h. BSMC proliferation was determined by Premixed WST-1 Cell Proliferation Reagent (Clontech Laboratories Inc., Mountain View, CA) in accord with the manufacturer's instructions.

Cell Migration Assay. The cell migration assay was carried out using the QCM Chemotaxis 8 μm cell migration assay system (Chemicon, Temecula, CA; Millipore Corp, Bedford, MA) according to the manufacturer's instructions.

Cells were seeded into the migration chamber. Control medium, various condition media, IL-8 depletion condition media, RANTES depletion condition media, or medium containing recombinant human interleukin-8 (rhIL-8) (20 ng/mL), recombinant human RANTES (rhRANTES) (20 ng/mL), and rhIL-8 plus rhRANTES (20 ng/mL + 20 ng/mL) was placed in the lower chamber. After 24 h of cell migration, cells that had migrated through the membrane were stained, lysed, and quantified on a microplate at 560 nm.

Real-Time RT-PCR and Microarray. RNA isolation was performed using the TRIzol reagent (Invitrogen). cDNA was prepared using an oligo(dT) primer and reverse transcriptase (Takara, Shiga, Japan) following standard protocols. Real-time PCR was performed using SYBR Green on the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Each PCR reaction mixture contained 200 nM of each primer, 10 μL of 2× SYBR Green PCR Master Mix (Applied Biosystems), and 5 μL of cDNA and RNase-free water in a total volume of 20 μL. The PCR reaction was carried out with a denaturation step at 95 °C for 10 min and then for 40 cycles at 95 °C for 15 s and 60 °C for 1 min. All PCRs were performed in triplicate and normalized to internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Relative expression was presented using the $2^{(\text{Ct reference sample} - \text{Ct experimental sample})}$ method.

Microarray experiment procedures were carried out following the manufacturer's protocols. Total RNA (1 μg) was amplified by an Agilent Quick Amp Labeling Kit (Agilent Technologies). DBP-BEAS-2B-CM-treated BSMC RNA was labeled by Cy5, and control-CM-treated BSMC RNA was labeled by Cy3 in an in vitro transcription process. Cy-labled cRNA (0.825 μg) was cleaved to an average size of about 50−100 nucleotides by incubation with fragmentation buffer (Agilent Technologies) at 60 °C for 30 min. Equal Cy-labled cRNA was pooled and hybridized to Agilent Whole Human Genome 4x44k oligo microarray (Agilent Technologies) at 65 °C for 17 h. After washing and drying by nitrogen gun blowing, microarrays were scanned by an Agilent microarray scanner (Agilent Technologies) at 535 nm for Cy3 and at 625 nm for Cy5. Scanned images were analyzed by Feature extraction software 10.5 (Agilent Technologies), image analysis and normalization software used to quantify signal and background intensity for each feature, and the data were substantially normalized by the rank-consistency-filtering LOWESS method.

Statistical analysis. Data were expressed as the mean ± SD. Statistical comparisons of the results were made using analysis of variance (ANOVA). Significant differences ($p < 0.05$) between the means of the two test groups were analyzed by Dunnett's test.

RESULTS

Condition Medium of Phthalate Ester-Treated BEAS-2B and HBE135-E6E7 (HBE) Increased Proliferation and Migration of BSMC. Increase in the mass of BSMC and decrease in the distance between BSMC and bronchial epithelia cells are important features of the remodeled wall in asthmatic airways.[8] These changes in BSMC can be induced by many factors produced by epithelial cells.[8] We harvested the condition medium of BEAS-2B and HBE cells that were precultured with butylbenzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), dibutyl phthalate (DBP), and diethyl phthalate (DEP) at the same concentration of 5 μM for 6 h (BBP-, BEHP-, DBP-, and DEP-BEAS-2B-CM and BBP-, BEHP-, DBP-, and DEP-HBE-CM) and then assessed the effects of condition media on the proliferation and migration of BSMC. As shown in Figure 2A, all eight epithelial cell condition media (BBP-, BEHP-, DBP-, and DEP-BEAS-2B-CM and BBP-, BEHP-, DBP-, and DEP-HBE-CM) increased the proliferation of BSMC after 72 h of treatment. Furthermore, all condition media increased the migration of BSMC after a 24 h attraction

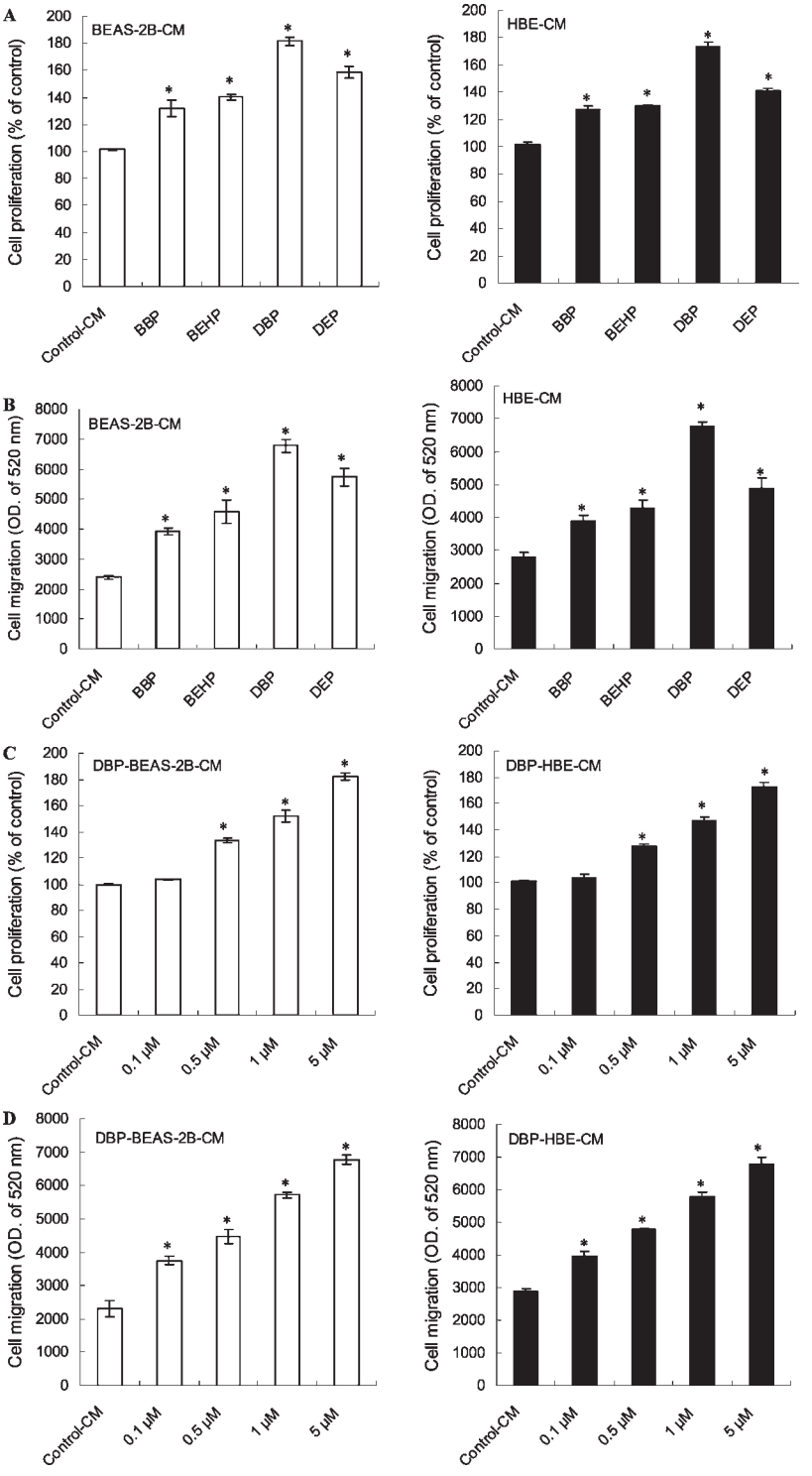

Figure 2. Condition medium (CM) of phthalate ester-treated BEAS-2B and HBE135-E6E7 (HBE) increased proliferation and migration of bronchial smooth muscle cells (BSMC). Butylbenzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), dibutyl phthalate (DBP), and diethyl phthalate (DEP) derived BEAS-2B-CM and HBE-CM increased BSMC proliferation (A) and migration (B). (C) DBP-BEAS-2B-CM and DBP-HBE-CM increased BSMC proliferation in a dose-dependent manner. (D) DBP-BEAS-2B-CM and DBP-HBE-CM enhance the migratory ability of BSMC in a concentration-dependent manner. BEAS-2B and HBE cells (2×10^6 cells/100 mm dish) were treated with vehicle control and 5 μM BBP, BEHP, DBP, or DEP for 6 h. The medium was replaced with fresh medium, and then cells were harvested after 24 h of incubation. The collected medium was defined as BBP-, BEHP-, DBP-, or DEP-BEAS-2B-CM and BBP-, BEHP-, DBP-, or DEP-HBE-CM. The effect of BBP-, BEHP-, DBP-, or DEP-BEAS-2B-CM and BBP-, BEHP-, DBP-, or DEP-HBE-CM on BSMC proliferation was assessed by WST-1 after 72 h of incubation. BSMC migration was assessed by QCM Chemotaxis cell migration assay system. The asterisk indicates a significant difference between control and test groups, $* p < 0.05$.

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Table 1. Two-fold Up-regulation Genes in BSMC after DBP-BEAS-2B-CM Treatment

gene name	description	fold
IL-8	*Homo sapiens* interleukin 8 (IL8),	7.531
NPTX1	*Homo sapiens* neuronal pentraxin I (NPTX1)	7.051
CXCL5	*Homo sapiens* chemokine (C-X-C motif) ligand 5	6.524
IGFBP1	*Homo sapiens* insulin-like growth factor binding protein 1 (IGFBP1)	4.307
SERPINB2	*Homo sapiens* serpin peptidase inhibitor	4.159
CYP1A1	*Homo sapiens* cytochrome P450, family 1, subfamily A, polypeptide 1	4.492
CYP1B1	*Homo sapiens* cytochrome P450, family 1, subfamily B, polypeptide 1	3.937
SHISA2	*Homo sapiens* shisa homologue 2 (*Xenopus laevis*)	3.621
STRA6	*Homo sapiens* stimulated by retinoic acid gene 6 homologue	3.230
FAM167A	*Homo sapiens* family with sequence similarity 167	3.162
CXCL2	*Homo sapiens* chemokine (C-X-C motif) ligand 2	3.034
DHRS3	*Homo sapiens* dehydrogenase/reductase (SDR family) member 3	2.886
CXCL3	*Homo sapiens* chemokine (C-X-C motif) ligand 3	2.473
IL-6	*Homo sapiens* interleukin 6 (IL6)	2.461
DDX58	*Homo sapiens* DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	2.435
APP	*Homo sapiens* amyloid β (A4) precursor protein (APP)	2.419
STC2	*Homo sapiens* stanniocalcin 2	2.382
TIPARP	*Homo sapiens* TCDD-inducible poly(ADP-ribose) polymerase	2.363
PARP14	*Homo sapiens* poly (ADP-ribose) polymerase family, member 14	2.239
RTP4	*Homo sapiens* receptor (chemosensory) transporter protein 4	2.254
AP2B1	*Homo sapiens* adaptor-related protein complex 2, β 1 subunit	2.138
KRTAP1−5	*Homo sapiens* keratin associated protein 1−5	2.080
ICAM	*Homo sapiens* intercellular adhesion molecule 1	2.032
MX2	*Homo sapiens* myxovirus (influenza virus) resistance 2 (mouse)	2.072
IL-1 β	*Homo sapiens* interleukin 1, β	2.001

(Figure 2B). The effect of DBP on both the induction of cell proliferation and migration is greater than that of BBP-, BEHP-, or DEP-treated epithelial cell CM. Because DBP appeared to have the strongest effect in increasing BSMC proliferation and migration, we selected DBP as the model to investigate the mechanism of phthalate esters in airway remodeling. Therefore, we consequently treated different concentrations of DBP and discovered that DBP-BEAS-2B-CM and DBP-HBE-CM increased the proliferation of BSMC in a dose-dependent manner after 72 h. Furthermore, DBP-BEAS-2B-CM and DBP-HBE-CM increased the migration of BSMC in a concentration-dependent manner (Figure 2C,D).

DBP-BEAS-2B-CM and DBP-HBE-CM Increased the Expression of Inflammatory, Adherence, and Chemotaxis Factors. Increased inflammatory response of BSMC is a cardinal feature in the development of airway remodeling.[15] We assessed whether DBP increased the inflammatory response through cross-talk of the epithelium and BSMC. As shown in Table 1, the 2-fold gene expressions are listed, as determined by microarray. DBP-BEAS-2B-CM increased the transcription of inflammatory cytokines, including IL-6, IL-8, CXCL2, CXCL3, CXCL5, and ICAM (Figure 3A). The data were also confirmed by real-time PCR in both DBP-BEAS-2B-CM- and DBP-HBE-CM-treated BSMC (Figure 3B). Similarly, DBP-BEAS-2B-CM and DBP-HBE-CM also increased the amount of IL-8 and CXCL5 on protein levels (Figure 3C,D).

DBP Caused an Inflammatory Response in Human Bronchial Epithelial Cells. Increase of inflammatory response in the epithelium plays an important role in the development of asthma.[16] We assessed the effect of DBP on various inflammatory cytokines in human bronchial epithelial cell lines BEAS-2B and HBE135-E6E7 (HBE). As shown in Figure 4A,B, DBP increased the production of IL-8 and RANTES in both BEAS-2B and HBE cell lines in a dose-dependent manner after 6 h of treatment. In contrast, DBP failed to affect the secretion of IL-6, CXCL5, and eotaxin (Figure 4C−E).

IL-8 and RANTES Are Major Factors of DBP-BEAS-2B-CM and DBP-HBE-CM in Increasing BSMC Proliferation and Migration. To address the role of IL-8 and RANTES in DBP-BEAS-2B-CM- and DBP-HBE-CM-mediated BSMC proliferation and migration, we depleted IL-8, RANTES, or both IL-8 and RANTES from DBP-BEAS-2B-CM and DBP-HBE-CM. The successful depletion of IL-8 and RANTES from DBP-BEAS-2B-CM and DBP-HBE-CM was confirmed by IL-8 and RANTES ELISA kits (data not shown). As shown in Figure 5A, IL-8 or RANTES depletion only partially inhibited BSMC proliferation caused by DBP-BEAS-2B-CM and DBP-HBE-CM. In contrast, depleting both IL-8 and RANTES completely reversed cell growth of BSMC presenting upon exposure to DBP-BEAS-2B-CM and DBP-HBE-CM. Similarly, increase of BSMC migration by DBP-BEAS-2B-CM and DBP-HBE-CM was totally abrogated upon depletion of both IL-8 and RANTES, whereas depletion of either IL-8 or RANTES alone only partially reversed BSMC migration (Figure 5B).

We also assessed the effect of recombinant human interleukin-8 (rhIL-8) and recombinant human RANTES (rhRANTES) on the proliferation and migration of BSMC. The results showed that both rhIL-8 and rhRANTES increase BSMC proliferation. Furthermore, the combination of rhIL-8 and rhRANTES caused a synergistic enhancement of BSMC proliferation (Figure 5C).

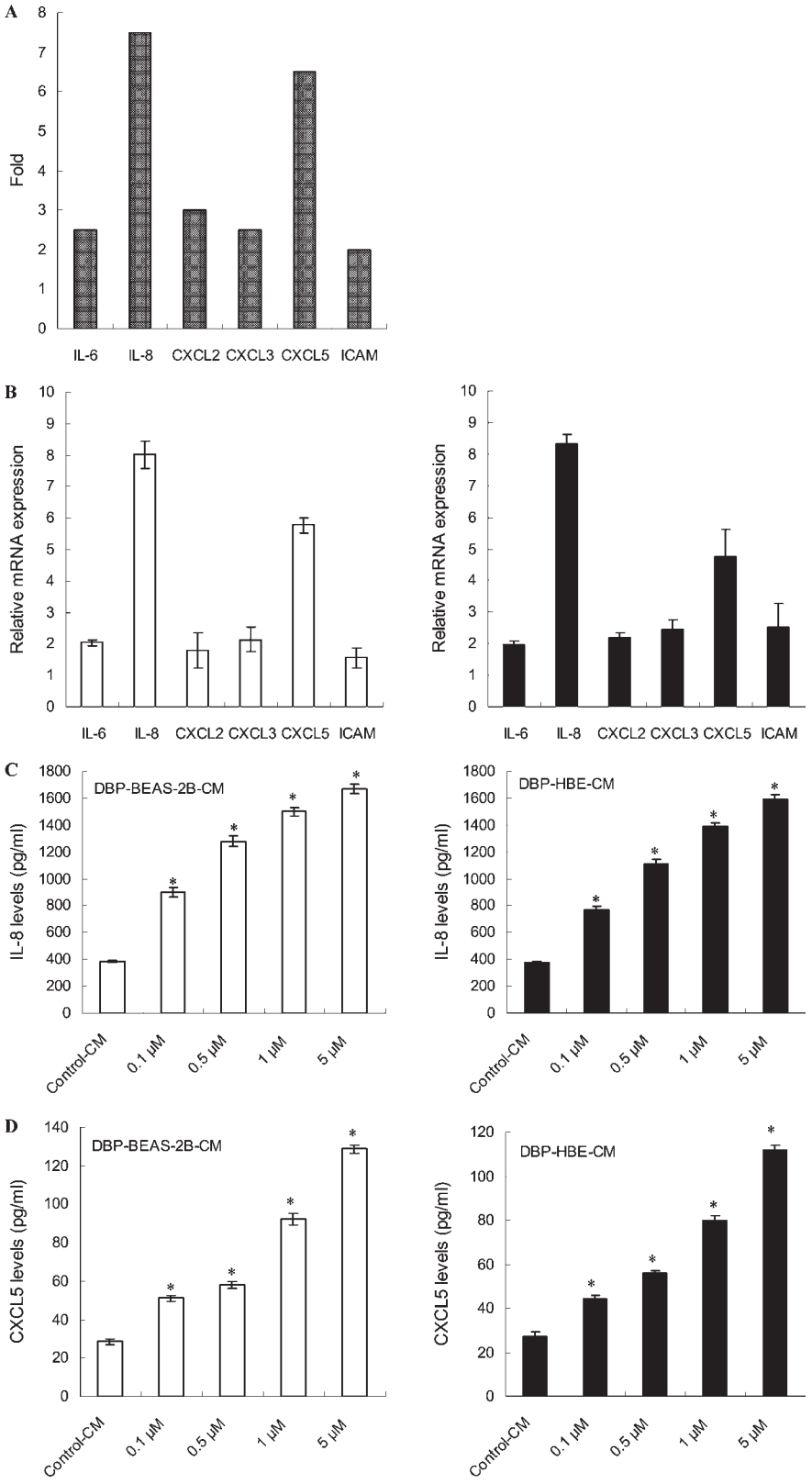

Figure 3. DBP-BEAS-2B-CM and DBP-HBE-CM increased the expression of inflammatory and chemotaxis factors in BSMC. DBP (5 μM)-BEAS-2B-CM and DBP (5 μM)-HBE-CM increased the expression of inflammatory factors and chemotaxis in mRNA levels, as assessed by microarray (A) and real-time PCR (B). DBP (5 μM)-BEAS-2B-CM and DBP (5 μM)-HBE-CM also increased the amounts of IL-8 (C) and CXCL5 (D) in protein levels. BSMC were treated with DBP-BEAS-2B-CM and DBP-HBE-CM for 6 h, and mRNA expression assessed by microarray and real-time PCR. For panels C and D, BSMC were treated with DBP-BEAS-2B and DBP-HBE-CM for 6 h, and the various amounts of proteins were detected by ELISA kit. The asterisk indicates a significant difference between control and test groups, $* p < 0.05$.

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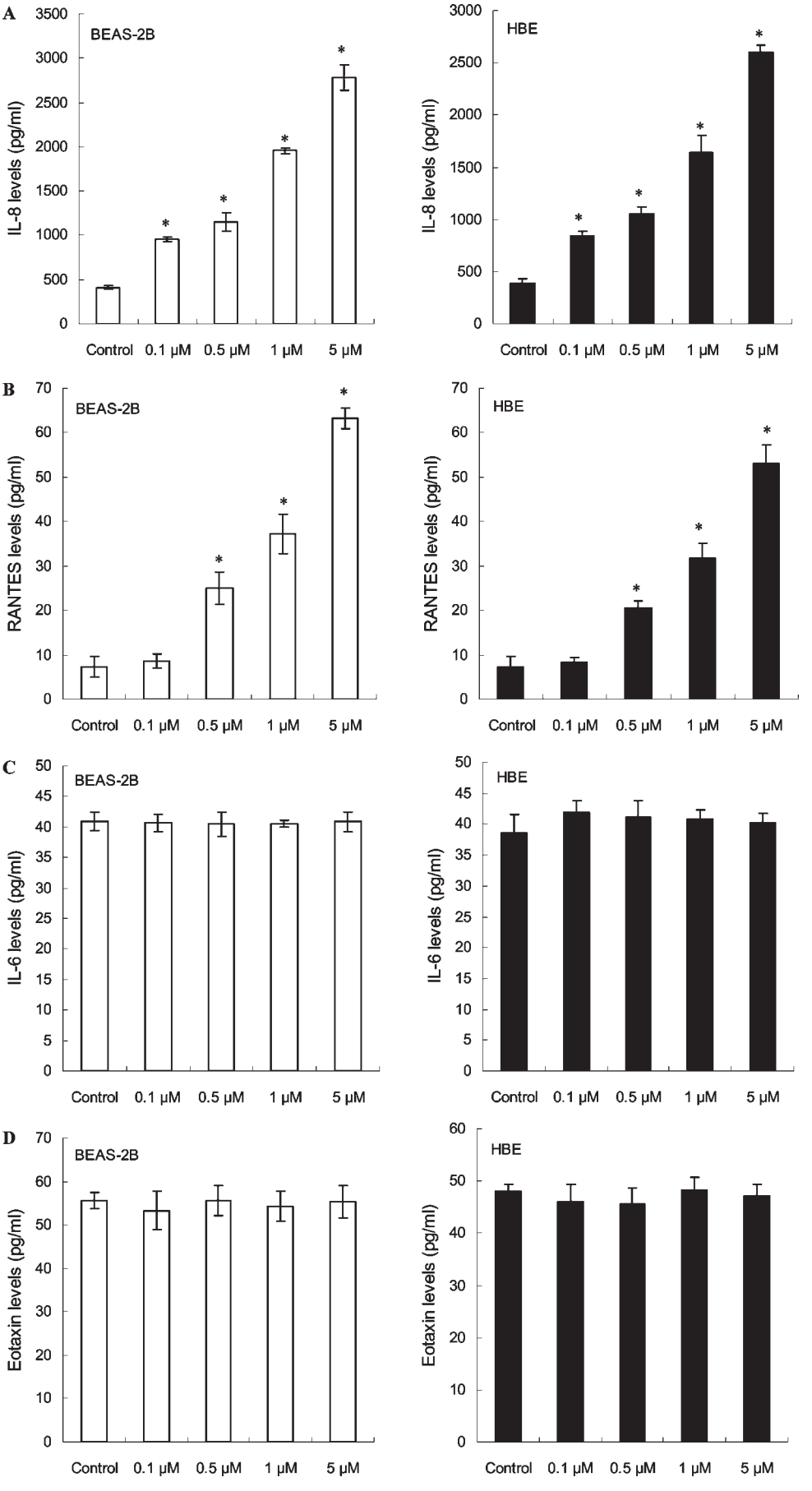

Figure 4. Continued

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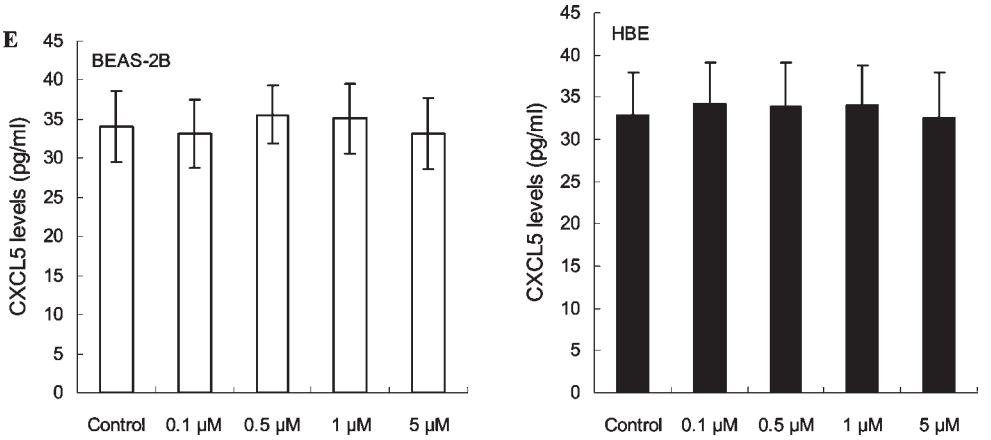

Figure 4. DBP caused an inflammatory response in human bronchial epithelial cells: levels of IL-8 (A) and RANTES (B) in DBP-treated BEAS-2B and HBE cell lines. DBP did not affect the levels of IL-6 (C), eotaxin (D), and CXCL5 (E) in bronchial epithelial cells. BEAS-2B and HBE cells were treated with various concentrations of DBP for 6 h. The supernatants were collected and the various cytokine levels assessed by ELISA kit. The asterisk indicates a significant difference between control and test groups, $* p < 0.05$.

Similarly, both rhIL-8 and rhRANTES increased the BSMC migration ability, and this effect was further enhanced when rhIL-8 was combined with rhRANTES to act as chemotaxis agent for BSMC (Figure 5D).

Ginger Suppresses DBP-Response IL-8 and RANTES in Mediating Airway Remodeling. The physiological and pathological contribution of BSMC is critical to the process of airway tissue remodeling.[17−19] BSMC proliferation (hyperplasia), increase in BSMC size (hypertrophy), and migration of BSMC toward the epithelium were clearly demonstrated in instances of fatal asthma attacks.[19,20] Because phthalate ester-induced BSMC proliferation and migration have been demonstrated, the search for a potential antidote in the fight against phthalate ester-induced asthma has become a matter of great urgency. We therefore assessed the effects of [6]-shogaol, [6]-gingerol, [8]-gingerol, and [10]-gingerol, which are major bioactive compounds present in *Z. officinale*, on DBP-induced airway remodeling. As shown in Figure 6A, epithelial cell condition media (DBP-BEAS-2B-CM and DBP-HBE-CM) increased the proliferation of BSMC after 72 h of treatment, whereas [6]-shogaol, [6]-gingerol, [8]-gingerol, or [10]-gingerol treatment decreased the proliferation of BSMC. Of these compounds, [6]-shogaol completely reversed cell growth of BSMC present upon exposure to DBP-BEAS-2B-CM and DBP-HBE-CM. Similarly, increase of BSMC migration triggered by DBP-BEAS-2B-CM and DBP-HBE-CM was totally abrogated upon [6]-shogaol treatment, whereas treatment with [6]-gingerol, [8]-gingerol, or [10]-gingerol alone only partially reversed BSMC migration (Figure 6B).

DISCUSSION

The present study is the first to investigate the interaction of airway epithelium and smooth muscle cells after exposure to phthalate esters. BBP, BEHP, DBP, and DEP caused human bronchial epithelial cell lines BEAS-2B and HBE135-E6E7 (HBE) to secrete IL-8 and RANTES, which enhance the proliferation and migration of human BSMC. This study is also the first to investigate the effect of ginger in reversing phthalate ester-mediated airway remodeling. Our findings suggest that ginger is capable of preventing phthalate ester-associated asthma.

Asthmatic airway remodeling is the pathophysiological modification of the normal airway wall structure, including changes in the composition and organization of its cellular and molecular constituents. These modifications are the major cause of the symptoms associated with decreased pulmonary function.[19] BSMC proliferation (hyperplasia), increase in BSMC size (hypertrophy), migration of BSMC toward the epithelium, and decreased rates of BSMC apoptosis all contribute to the increase in airway smooth muscle mass, a hallmark feature of tissue remodeling. This increase in BSMC mass has been reported to correlate with the severity of asthma.[19−23] The bronchial epithelium is known to play a critical regulatory role in the maintenance of airway function and integrity[24,25] and can produce various factors and cytokines/chemokines to regulate other systems or tissues, including BSMC.[8,26] Our results show that phthalate esters BBP, BEHP, DBP, and DEP caused epithelial cells to produce inflammatory cytokines IL-8 and RANTES, which subsequently induced BSMC proliferation and migration. Depleting both IL-8 and RANTES completely reversed the effect of DBP-BEAS-2B-CM- and DBP-HBE-CM-mediated BSMC proliferation and migration, suggesting this effect is a synergistic influence of IL-8 and RANTES. However, this effect did not take place when either IL-8 or RANTES alone was depleted. This is an important correlation to our finding on the clinical significance of elevated IL-8 and RANTES levels in asthma patients.

Airway inflammation is a major factor in the pathogenesis of asthma,[8,9] and corticosteroids are currently the most effective anti-inflammatory therapy for persistent asthma. Their efficacy in controlling airway inflammation in asthma has been well documented;[19,27] however, their effect on airway remodeling is still debatable. Although some studies have suggested a modest effect of corticosteroids on airway remodeling, the majority of the literature suggests that corticosteroids have little or no effect on tissue remodeling.[19,27−29] Our results show that [6]-shogaol, [6]-gingerol, [8]-gingerol, and [10]-gingerol, which are the major bioactive compounds present in *Z. officinale*, could reverse DBP-induced BSMC proliferation and migration. Therefore, we propose ginger suppresses DBP-response IL-8 and RANTES in mediating airway remodeling.

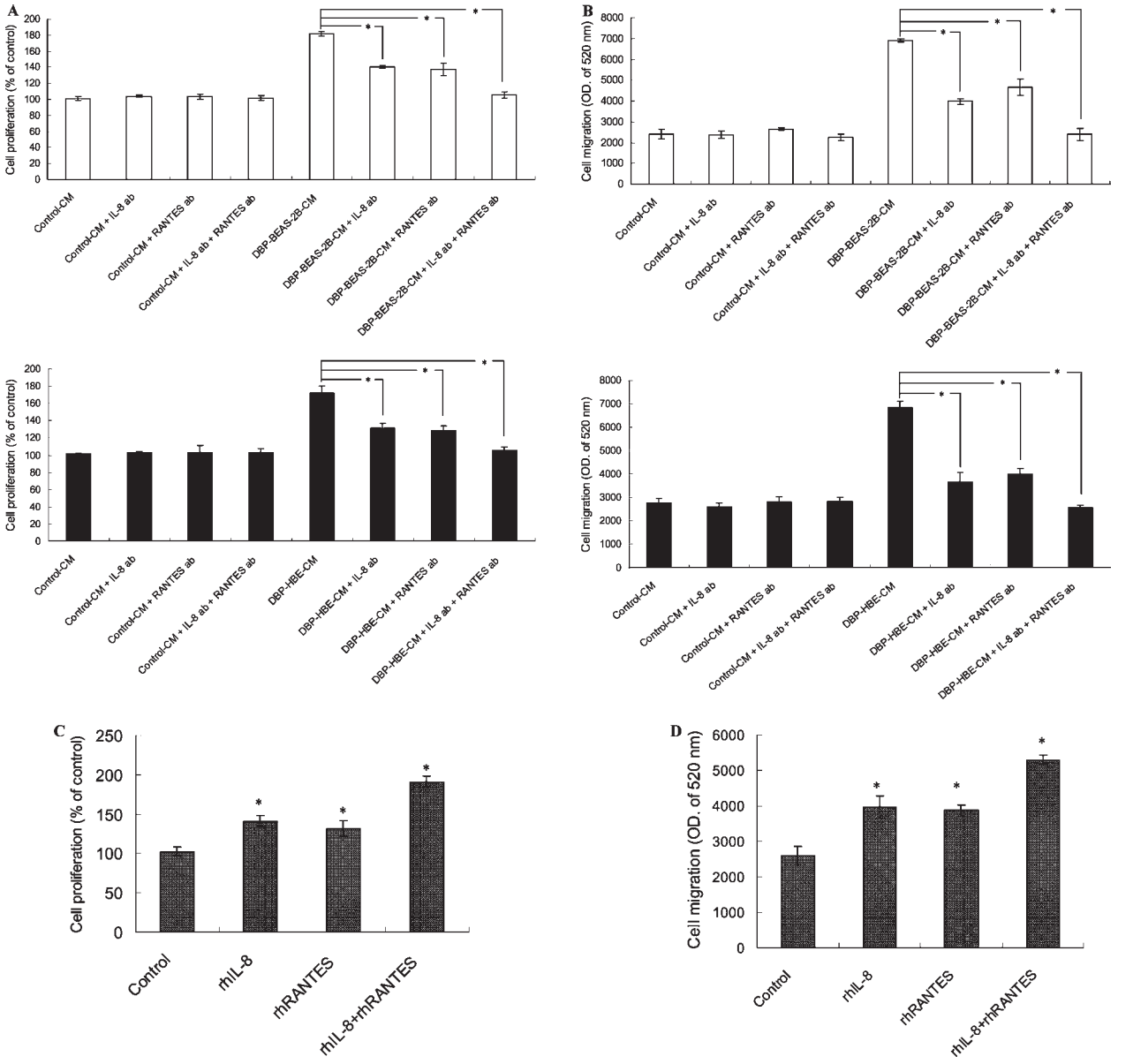

Figure 5. IL-8 and RANTES are major factors of DBP-BEAS-2B-CM and DBP-HBE-CM in increasing BSMC proliferation and migration. (A) The proliferation of BSMC present in IL-8 and/or RANTES depleted DBP (5 μM)-BEAS-2B-CM and DBP (5 μM)-HBE-CM. (B) The migration of BSMC presenting in IL-8 and/or RANTES depleted DBP-BEAS-2B-CM and DBP-HBE-CM. Recombinant human interleukin-8 (rhIL-8) and/or recombinant human RANTES (rhRANTES) increased proliferation (C) and migration (D) in BSMC. IL-8 and RANTES depletion from DBP-BEAS-2B-CM and DBP-HBE-CM was performed using anti-IL-8 and anti-RANTES antibodies (2 μg/mL) and Sepharose A/G beads, following regular immunoprecipitation techniques. The depletion of various cytokines from DBP-BEAS-2B-CM and DBP-HBE-CM and the effect on BSMC proliferation were assessed by WST-1 after 72 h of incubation. BSMC migration was assessed by QCM Chemotaxis cell migration assay system. All experiments were performed independently at least three times. The asterisk indicates a significant difference between two test groups, $* p < 0.05$.

In this study, we found that [6]-shogaol was the strongest inhibitor of DBP-mediated BSMC proliferation and migration. This finding is similar to those of other studies with regard to the anticancer, antioxidant, and anti-inflammatory effects of [6]-gingerol.[12,30] The α,β-unsaturated carbonyl group in [6]-shogaol might influence the conformation of the molecule and modulate its inhibitory effect, and this hypothesis could provide new information for the design of antiasthmatic airway remodeling agents and future study of these functional groups.[12,30]

Our findings indicate that condition media from phthalate ester-treated epithelial cells stimulate BSMC's proliferation and migration. IL-8 and RANTES in the condition medium resulted in an enhanced effect on BSMC growth and movement. Because anti-inflammatory medications have limited impact on airway remodeling,[19,27−29] determining the mechanisms involved in remodeling, including the role of structural cells in asthma, might open new horizons for more effective therapeutic interventions.[19] This is the first investigation to provide evidence that ginger has great potential as a

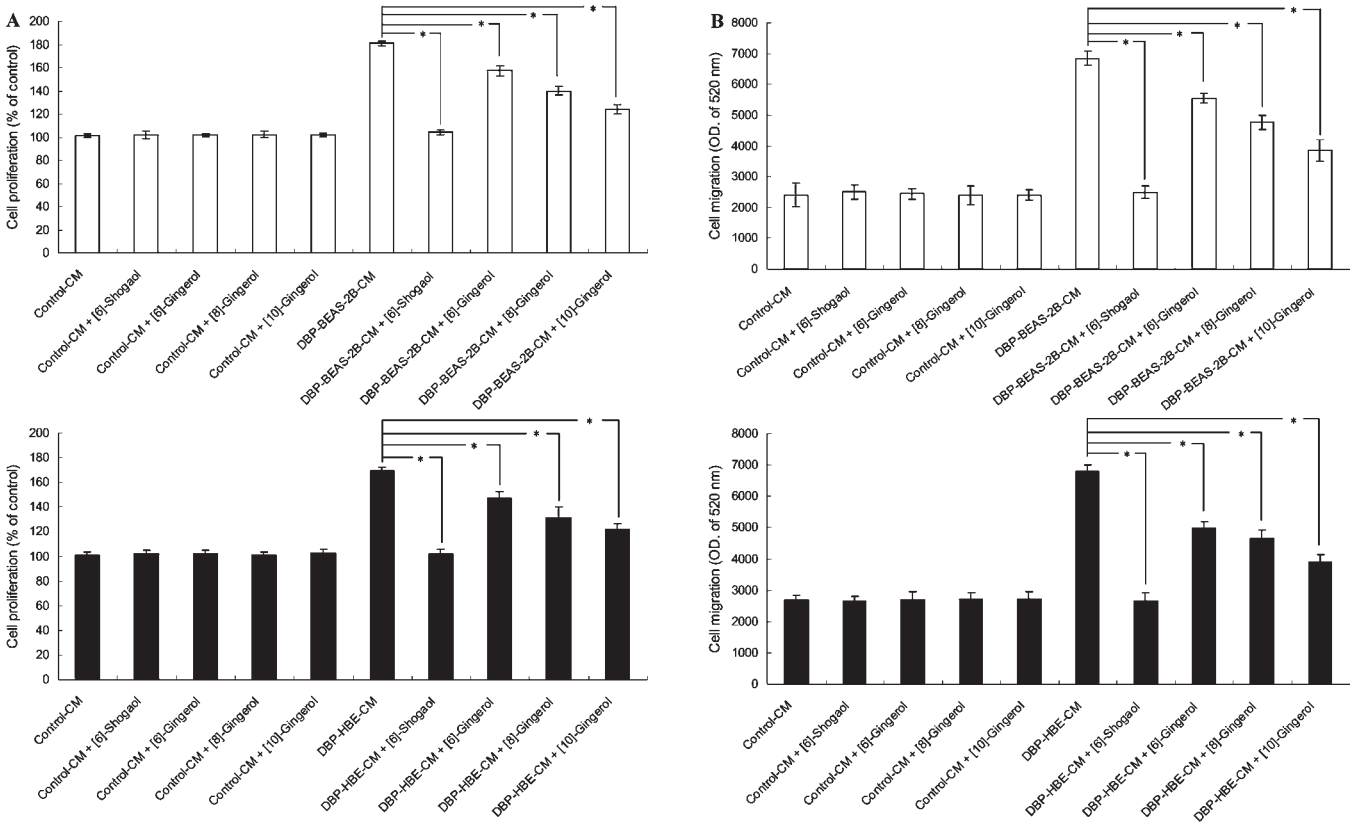

Figure 6. Ginger suppresses DBP-mediating airway remodeling. [6]-Shogaol, [6]-gingerol, [8]-gingerol, and [10]-gingerol reversed cell proliferation (A) and migration (B) of BSMC presenting in exposure to DBP-BEAS-2B-CM and DBP-HBE-CM. BSMC were pretreated with or without [6]-gingerol, [8]-gingerol, [10]-gingerol, or [6]-shogaol (5 μM) for 1 h, and then control-CM, DBP-BEAS-2B-CM, or DBP-HBE-CM, with or without different ginger compounds, was added for another 72 h. Cell proliferation was assessed by WST-1 assay. BSMC migration was assessed by QCM Chemotaxis cell migration assay system. BSMC cells were seeded into the top chamber and treated with or without [6]-gingerol, [8]-gingerol, [10]-gingerol, or [6]-shogaol (5 μM). Control-CM, DBP-BEAS-2B-CM, or DBP-HBE-CM was added to the bottom wells for 24 h as chemoattractant. Migratory cells were poststained with CyQuant GR dye and examined using a fluorescence plate reader at excitation/emission wavelengths of 485/530 nm. The asterisk indicates a significant difference between two test groups, $* p < 0.05$.

novel antiasthmatic airway remodeling agent in the prevention and treatment of asthma.

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REFERENCES

(1) Martino-Andrade, A. J.; Chahoud, I. Reproductive toxicity of phthalate esters. *Mol. Nutr. Food Res.* **2010**, *54*, 148–157.

(2) Ge, R. S.; Chen, G. R.; Tanrikut, C.; Hardy, M. P. Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations. *Reprod. Toxicol.* **2007**, *23*, 366–373.

(3) Jaakkola, J. J.; Parise, H.; Kislitsin, V.; Lebedeva, N. I.; Spengler, J. D. Asthma, wheezing, and allergies in Russian schoolchildren in relation to new surface materials in the home. *Am. J. Public Health* **2004**, *94*, 560–562.

(4) Wang, T. N.; Lin, M. C.; Wu, C. C.; Leung, S. Y.; Huang, M. S.; Chuang, H. Y.; Lee, C. H.; Wu, D. C.; Ho, P. S.; Ko, A. M.; Chang, P. Y.; Ko, Y. C. Risks of exposure to occupational asthmogens in atopic and nonatopic asthma: a case-control study in taiwan. *Am. J. Respir. Crit. Care Med.* **2010**, *182*, 1369–1376.

(5) Bornehag, C. G.; Nanberg, E. Phthalate exposure and asthma in children. *Int. J. Androl.* **2010**, *33*, 333–345.

(6) Krug, N.; Rabe, K. F. Animal models for human asthma: the perspective of a clinician. *Curr. Drug Targets* **2008**, *9*, 438–442.

(7) Kumar, R. K.; Herbert, C.; Foster, P. S. The "classical" ovalbumin challenge model of asthma in mice. *Curr. Drug Targets* **2008**, *9*, 485–494.

(8) Benayoun, L.; Druilhe, A.; Dombret, M. C.; Aubier, M.; Pretolani, M. Airway structural alterations selectively associated with severe asthma. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1360–1368.

(9) Dekkers, B. G.; Maarsingh, H.; Meurs, H.; Gosens, R. Airway structural components drive airway smooth muscle remodeling in asthma. *Proc. Am. Thorac. Soc.* **2009**, *6*, 683–692.

(10) Rahuman, A. A.; Gopalakrishnan, G.; Venkatesan, P.; Geetha, K.; Bagavan, A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. *Phytother. Res.* **2008**, *22*, 1035–1039.

(11) Ali, B. H.; Blunden, G.; Tanira, M. O.; Nemmar, A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food Chem. Toxicol.* **2008**, *46*, 409–420.

(12) Dugasani, S.; Pichika, M. R.; Nadarajah, V. D.; Balijepalli, M. K.; Tandra, S.; Korlakunta, J. N. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *J. Ethnopharmacol.* **2010**, *127*, 515–520.

(13) Pan, M. H.; Hsieh, M. C.; Hsu, P. C.; Ho, S. Y.; Lai, C. S.; Wu, H.; Sang, S.; Ho, C. T. 6-Shogaol suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. *Mol. Nutr. Food Res.* **2008**, *52*, 1467–1477.

(14) Pan, M. H.; Hsieh, M. C.; Kuo, J. M.; Lai, C. S.; Wu, H.; Sang, S.; Ho, C. T. 6-Shogaol induces apoptosis in human colorectal carcinoma cells via ROS production, caspase activation, and GADD 153 expression. *Mol. Nutr. Food Res.* **2008**, *52*, 527–537.

(15) Tliba, O.; Panettieri, R. A., Jr. Regulation of inflammation by airway smooth muscle. *Curr. Allergy Asthma Rep.* **2008**, *8*, 262–268.

(16) Holgate, S. T.; Roberts, G.; Arshad, H. S.; Howarth, P. H.; Davies, D. E. The role of the airway epithelium and its interaction with environmental factors in asthma pathogenesis. *Proc. Am. Thorac. Soc.* **2009**, *6*, 655–659.

(17) Fernandes, D. J.; Mitchell, R. W.; Lakser, O.; Dowell, M.; Stewart, A. G.; Solway, J. Do inflammatory mediators influence the contribution of airway smooth muscle contraction to airway hyperresponsiveness in asthma?. *J. Appl. Physiol.* **2003**, *95*, 844–853.

(18) Ma, X.; Cheng, Z.; Kong, H.; Wang, Y.; Unruh, H.; Stephens, N. L.; Laviolette, M. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2002**, *283*, L1181–L1189.

(19) Halwani, R.; Al-Muhsen, S.; Hamid, Q. Airway remodeling in asthma. *Curr. Opin. Pharmacol.* **2010**, *10*, 236–245.

(20) Ebina, M.; Takahashi, T.; Chiba, T.; Motomiya, M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am. Rev. Respir. Dis.* **1993**, *148*, 720–726.

(21) Johnson, P. R.; Roth, M.; Tamm, M.; Hughes, M.; Ge, Q.; King, G.; Burgess, J. K.; Black, J. L. Airway smooth muscle cell proliferation is increased in asthma. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 474–477.

(22) Martin, J. G.; Ramos-Barbón, D. Airway smooth muscle growth from the perspective of animal models. *Respir. Physiol. Neurobiol.* **2003**, *137*, 251–261.

(23) Madison, J. M. Migration of airway smooth muscle cells. *Am. J. Respir. Cell Mol. Biol.* **2003**, *29*, 8–11.

(24) Makinde, T.; Murphy, R. F.; Agrawal, D. K. The regulatory role of TGF-β in airway remodeling in asthma. *Immunol. Cell Biol.* **2007**, *85*, 348–356.

(25) Davies, D. E. The role of the epithelium in airway remodeling in asthma. *Proc. Am. Thorac. Soc.* **2009**, *6*, 678–682.

(26) Bossé, Y.; Thompson, C.; McMahon, S.; Dubois, C. M.; Stankova, J.; Rola-Pleszczynski, M. Leukotriene D4-induced, epithelial cell-derived transforming growth factor beta1 in human bronchial smooth muscle cell proliferation. *Clin. Exp. Allergy* **2008**, *38*, 113–121.

(27) Kelly, M. M.; O'Connor, T. M.; Leigh, R.; Otis, J.; Gwozd, C.; Gauvreau, G. M.; Gauldie, J.; O'Byrne, P. M. Effects of budesonide and formoterol on allergen-induced airway responses, inflammation, and airway remodeling in asthma. *J. Allergy Clin. Immunol.* **2010**, *125*, 349–356.e13.

(28) Molet, S. M.; Hamid, Q. A.; Hamilos, D. L. IL-11 and IL-17 expression in nasal polyps: relationship to collagen deposition and suppression by intranasal fluticasone propionate. *Laryngoscope* **2003**, *113*, 1803–1812.

(29) Chakir, J.; Shannon, J.; Molet, S.; Fukakusa, M.; Elias, J.; Laviolette, M.; Boulet, L. P.; Hamid, Q. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-β, IL-11, IL-17, and type I and type III collagen expression. *J. Allergy Clin. Immunol.* **2003**, *111*, 1293–1298.

(30) Wu, H.; Hsieh, M. C.; Lo, C. Y.; Liu, C. B.; Sang, S.; Ho, C. T.; Pan, M. H. 6-Shogaol is more effective than 6-gingerol and curcumin in inhibiting 12-*O*-tetradecanoylphorbol 13-acetate-induced tumor promotion in mice. *Mol. Nutr. Food Res.* **2010**, *54*, 1296–1306.