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Tumor necrosis factor superfamily member LIGHT induces epithelial–mesenchymal transition in A549 human alveolar epithelial cells

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ABSTRACT

Fibrosis is an abnormal response to organ injury, characterized by accumulation of activated fibroblasts at the sites of injury. Fibroblasts arise from several sources, including resident fibroblasts and circulating fibrocytes that infiltrate organ tissue. Recently, epithelial–mesenchymal transition (EMT) has been recognized as a source of mesenchymal cells. EMT is induced by various growth factors, such as transforming growth factor (TGF)-β1, and enhanced by inflammatory cytokines. Recently the tumor necrosis factor superfamily member LIGHT has been implicated in the pathogenesis of inflammatory disease and airway remodeling in severe asthma. We hypothesized that LIGHT might contribute to the pathogenesis of airway fibrosis via enhancement of EMT. Therefore, we investigated LIGHT's ability to induce EMT. A549 cells were stimulated with LIGHT, TGF-β1 or both for 48 h. To estimate EMT, we evaluated the expression of epithelial and mesenchymal markers using immunocytochemistry, Western blotting and quantitative RT-PCR. Signaling pathways for EMT were characterized by Western analysis to detect phosphorylation of Erk1/2 and smad2. LIGHT enhanced TGF-β1-induced EMT both morphologically, by suppressing E-cadherin and enhancing vimentin, and functionally, by enhancing cell contractility. Additionally, LIGHT induced EMT without TGF-β1. Evaluation of the mechanism showed that LIGHT did not induce TGF-β1 production or affect the smad-*snai1* pathway. Inhibition of Erk1/2 phosphorylation reduced LIGHT-induced EMT, indicating the Erk1/2 pathway to be a key pathway in LIGHT-induced EMT. In summary, LIGHT enhanced TGF-β1-induced EMT but also induced EMT via the Erk1/2 pathway by itself, without TGF-β1 signaling. LIGHT may contribute to the pathogenesis of airway fibrosis through enhancement of EMT.

1. Introduction

Fibrosis, that is, fibroblast activation with generation of provisional extracellular matrix (ECM), is an abnormal response of organs to injury, inflammation or stress [18]. Successful tissue repair relies on a balance between ECM synthesis and degradation, as well as re-epithelization of damaged epithelial surfaces. Abnormal tissue repair and fibrosis are often suggested to be associated with a variety of chronic progressive diseases, including pulmonary fibrosis, cardiovascular fibrosis, liver cirrhosis, end stage kidney disease, systemic sclerosis and autoimmune disease [26]. Idiopathic pulmonary fibrosis (IPF), which is the most common interstitial lung disease, is a devastating, progressive respiratory disease, with a mean survival of 2–3 years from initial diagnosis. To date, there is no effective treatment that shows reversal of disease progression and survival benefit [20]. A key histological feature of IPF is formation of fibroblastic foci that reflect sites of active, ongoing fibrogenesis. Increased numbers of fibroblastic foci have been associated with disease activity and a more rapid disease progression of IPF [13,19]. Lung fibroblasts arise from several sources, including resident pulmonary fibroblasts and circulating fibrocytes that infiltrate the lung. Recently, epithelial–mesenchymal transition (EMT), a process whereby epithelial cells undergo transition to a mesenchymal phenotype that gives rise to fibroblasts, has been recognized as a source of mesenchymal cells [6].

Abbreviations: EMT, epithelial–mesenchymal transition; LIGHT, homologous to lymphotoxins, exhibits inducible expression and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes; TNFSF, tumor necrosis factor superfamily; TGF, transforming growth factor; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; α-SMA, α-smooth muscle actin.

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EMT is an important process during fetal development and progression of cancer, such as tumor invasion and tumor metastasis. EMT permits epithelial cells to acquire the capacity to migrate by down-regulating epithelial markers, such as E-cadherin, and gaining expression of mesenchymal markers, such as vimentin and α-smooth muscle actin (SMA) [11,23]. EMT has been increasingly implicated in the pathogenesis of tissue fibrosis, such as in the kidney [3] and lung [29]. EMT can be induced by such growth factors as transforming growth factor (TGF)-β, fibroblast growth factor-2, epidermal growth factor and hepatocyte growth factor. These growth factors are associated with the tissue repair process and reported to be upregulated in chronic inflammatory disease. Additionally, chronic inflammation has been shown to promote fibrotic disease [21]. Several inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin-1β, were reported to enhance EMT [1,12,28]. Thus, chronic inflammation can contribute to tissue fibrosis through TGF-β-induced EMT enhanced by inflammatory cytokines.

The tumor necrosis factor superfamily member ligand LIGHT (TNFSF14; homologous to lymphotoxins, exhibits inducible expression and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes [17]) has been implicated in the pathogenesis of such inflammatory diseases as rheumatoid arthritis and inflammatory bowel disease [8,25]. Moreover, LIGHT is recognized as a key mediator in chronic airway inflammatory diseases, such as bronchial asthma, and it is associated with the disease severity of asthma [2,7]. Furthermore, LIGHT plays an important role in the pathogenesis of airway remodeling that is associated with TGF-β and IL-13 produced by inflammatory cells [2]. However, there are no data regarding a possible effect of LIGHT on EMT.

Taken together, EMT may contribute to tissue fibrosis and be increased in chronic inflammation. LIGHT is a key cytokine in airway inflammation and fibrosis. Thus, we hypothesized that LIGHT might contribute to the pathogenesis of airway fibrosis through enhancement of EMT. We first investigated whether LIGHT affected TGF-β1-induced EMT, causing morphological changes and acquisition of contractility, which is a property of mesenchymal cells. We then investigated whether LIGHT itself induced EMT. Finally, we examined the mechanisms of LIGHT-induced EMT, elucidating the intracellular signaling.

2. Materials and methods

2.1. Reagents

The details of the reagents are presented in the online supplement.

2.2. Cells and cell cultures

A549 cells were purchased from the American Type Culture Collection (Manassas, VA). The details of cell culture are provided in the online supplement.

2.3. Gel contraction assay

Cells were cast into collagen gels using previously reported methods. The details are provided in the online supplement.

2.4. Quantitative reverse transcriptional PCR (RT-PCR) analysis of messenger RNA

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Tokyo, Japan). The details of RT-PCR are provided in the online supplement. Individual data were normalized against the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Untreated control samples were set to 1.0, and the values of the fold change in expression following treatment were presented as bar graphs ± standard error of the mean.

2.5. Immunostaining of cultured cells

A549 cells were seeded into chamber slides (IWAKI), and immunocytochemical staining was performed using the streptavidin–biotin–peroxidase method (LSAB2 Kit/HRP; DAKO, Kyoto, Japan). The details are provided in the online supplement.

2.6. Western blot analysis

The details of the methods and antibodies are provided in the online supplement.

2.7. Antibodies

The antibodies (all purchased from Cell Signaling Technology, Beverly, MA) used were rabbit anti-human E-cadherin antibody #3195 1:3000, rabbit anti-vimentin antibody #5741 1:5000, rabbit anti-smad2 antibody #3122 1:2000, rabbit anti-phospho-smad2 antibody #3104 1:3000, rabbit anti-p44/42 mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (Erk1/2) antibody #9102 1:3000, rabbit anti-phospho-Erk1/2 antibody #9101 1:3000 and anti-rabbit IgG, HRP-linked antibody #7074 1:15,000.

Equal protein loading was confirmed by probing the blot with antibody against α-tubulin (Sigma–Aldrich) at a 1:5000 dilution and anti-mouse HRP-linked antibody at a 1:15,000 dilution.

2.8. Enzyme-linked immunosorbent assay (ELISA)

The concentration of TGF-β1 was estimated by using Quantikine ELISA human TGF-β1 immunoassay (R&D Systems #DB100B) according to the manufacturer's instructions. The details are provided in the online supplement.

2.9. Statistics

Results were confirmed by repeating experiments on at least three separate occasions. Data shown in the figures are pooled data for each experiment and expressed as the mean ± SEM. Analyses were performed using JMP (Version 9; SAS Institute Inc., Tokyo, Japan). Samples with multiple comparisons were analyzed for significance by analysis of variance (ANOVA). When ANOVA indicated significant differences between groups, Tukey–Kramer's HSD was applied. P values of <0.05 were considered to be significant.

3. Results

3.1. LIGHT enhances TGF-β1-induced EMT in A549 cells

A549 cells, a human alveolar epithelial cell line, were incubated with TGF-β1 (5 ng/ml) with/without LIGHT (10 ng/ml) for 48 h. Phase contrast images showed that the treated cells changed in morphology from a cobblestone appearance—a characteristic of epithelial cells—to a spindle-shaped appearance that is characteristic of mesenchymal cells. Immunocytochemical staining confirmed that the morphological changes were associated with loss of expression of an epithelial marker, E-cadherin, and acquisition of expression of a mesenchymal marker, vimentin (Supplemental Fig. 1). In addition, costimulation with TGF-β1 and LIGHT led to a

more spindle-like, elongated shape compared with stimulation with TGF-β1 alone, with less immunoreactivity for E-cadherin and more for vimentin. Immunoblots (Fig. 1A) showed that A549 cells underwent EMT, characterized by loss of E-cadherin and acquisition of vimentin. We used quantitative (q)-RT-PCR to confirm whether the morphological and immunoreactivity changes correlated with expression of epithelial and mesenchymal marker genes (Fig. 1B). A549 cells costimulated with TGF-β1 and LIGHT for 48 h showed significantly decreased expression of mRNA for E-cadherin and significantly increased expression of mRNA for vimentin compared with stimulation with TGF-β1 alone. Thus, LIGHT enhanced TGF-β1-induced EMT morphologically.

Next, we performed gel contraction assay to determine whether EMT-induced cells acquired contractility, a key property of mesen-

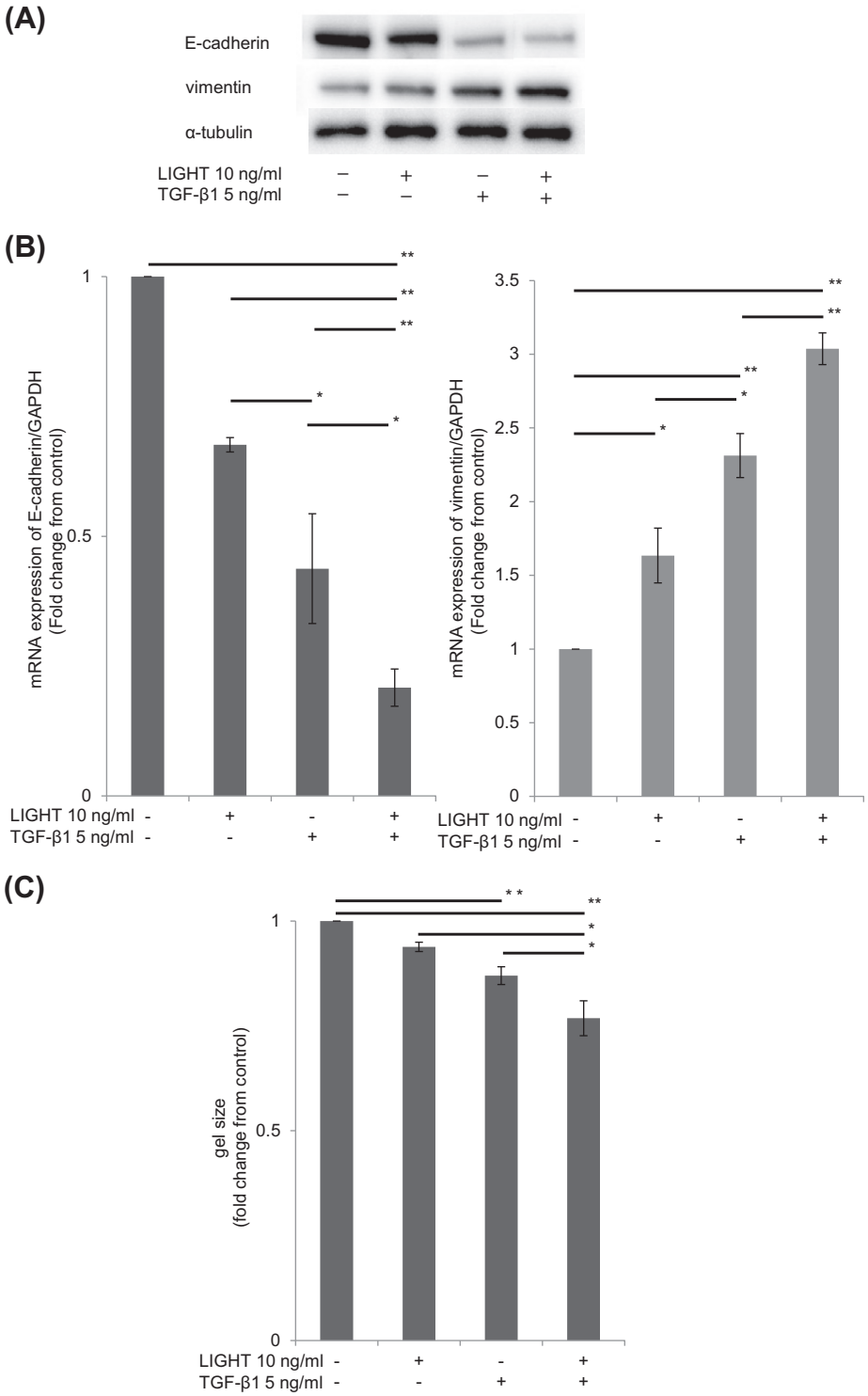

Fig. 1. LIGHT enhances TGF-β1-induced EMT in A549 cells. (A) Western blotting was performed for E-cadherin (upper images) and vimentin (lower images) in A549 cells treated with LIGHT (10 ng/ml) and/or TGF-β1 (5 ng/ml) for 48 h. (B) We determined the expression of mRNA for E-cadherin and vimentin in A549 cells stimulated with LIGHT (10 ng/ml) and/or TGF-β1 (5 ng/ml) for 48 h. E-cadherin mRNA expression was significantly decreased by the combination of LIGHT and TGF-β1 compared with TGF-β1 alone. $n = 5$ separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (C) We investigated the effects of LIGHT and/or TGF-β1 on collagen gel contraction mediated by A549 cells. Costimulation with LIGHT and TGF-β1 significantly decreased the gel size compared with TGF-β1 alone. $n = 6$ separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

chymal cells. The gel contraction assay—in which fibroblasts are cultured in three-dimensional gels of type I collagen—has been used as an ideal *in vitro* model of the contraction that characterizes both normal wound repair and fibrosis [4]. In the assay, mesenchymal cells are thought to attach to collagen fibers through integrin-dependent mechanisms and generate mechanical tension, which leads to tissue contraction and reduced size of collagen gels. Fig. 1C shows that stimulation with TGF-β1 significantly decreased the gel size compared with no stimulation. Costimulation with LIGHT and TGF-β1 significantly decreased the gel size compared with TGF-β1 alone. Thus, LIGHT enhanced the contractility seen in TGF-β1-induced EMT.

These results indicate that LIGHT enhanced TGF-β1-induced EMT and that cells that underwent EMT gained cell contractility, a mesenchymal cell property.

3.2. LIGHT induces EMT in A549 cells

Because we found that LIGHT enhanced TGF-β1-induced EMT, we further evaluated whether LIGHT itself could induce EMT. We examined for a concentration-dependent effect of LIGHT on the expression of cell surface markers associated with EMT. Stimulation with various concentrations (0.1–50 ng/ml) of LIGHT for 48 h induced EMT in a concentration-dependent manner, as evidenced by suppression of E-cadherin and expression of vimentin, N-cadherin and α-SMA (Fig. 2A). The expressions of E-cadherin and vimentin mRNA were quantified by q-RT-PCR. LIGHT significantly reduced the E-cadherin mRNA level while simultaneously increasing expression of vimentin, concentration-dependently (Fig. 2B).

3.3. Mechanisms of LIGHT-induced EMT in A549 cells

Since we found that LIGHT enhanced TGF-β1-induced EMT and itself induced EMT of A549 cells, we tried to elucidate the mechanisms of LIGHT-induced EMT.

3.3.1. LIGHT does not induce TGF-β1 production

First, we analyzed the effect of LIGHT on TGF-β1 production to determine whether LIGHT-induced EMT was due to production of TGF-β1, as an autocrine mechanism. We stimulated A549 cells

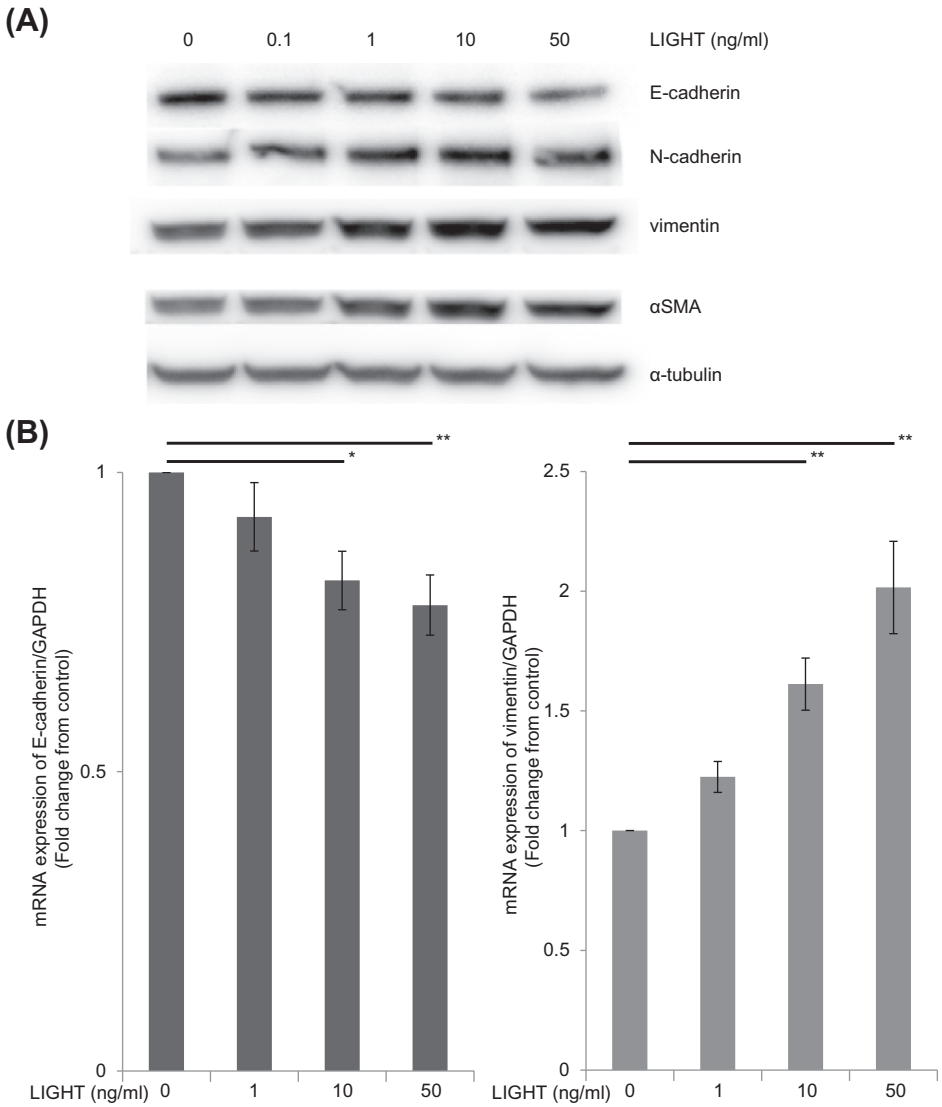

Fig. 2. LIGHT induces EMT in A549 cells. (A) We determined the expression of epithelial and mesenchymal markers in A549 cells. The cells were stimulated with various concentrations of LIGHT (0, 0.1, 1, 10, 50 ng/ml) for 48 h. LIGHT reduced expression E-cadherin (as simultaneously increasing expression of N-cadherin, vimentin and α-SMA (as mesenchymal markers), concentration-dependently. (B) We investigated the expression of mRNA for E-cadherin and vimentin in A549 cells stimulated with various concentrations of LIGHT (0, 1, 10, 50 ng/ml) for 48 h. LIGHT significantly reduced E-cadherin mRNA levels while simultaneously increasing expression of vimentin, concentrationdependently. $n = 6$ separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

with various concentrations of LIGHT (0, 10, 50 ng/ml) for 24 h, measured the concentration of TGF-β1 in the cell supernatant using an ELISA kit (Supplemental Fig. 2A) and examined the cells' expression of TGF-β1 mRNA by q-RT-PCR (Supplemental Fig. 2B). Neither the TGF-β1 concentration nor the TGF-β1 mRNA expression level differed significantly between the control and LIGHT-stimulated groups. Thus, LIGHT did not induce production of TGF-β1, which is the most potent known inducer of EMT, or enhance TGF-β1-induced EMT by an autocrine mechanism.

3.3.2. LIGHT does not affect the smad-snai1pathway

Next, since LIGHT enhanced TGF-β1-induced EMT, we examined the effect of LIGHT on the smad-*snai1* signaling pathway, which is one of the major pathways in TGF-β1-induced EMT [24]. We first evaluated phosphorylation of smad2 by immunoblot assay.

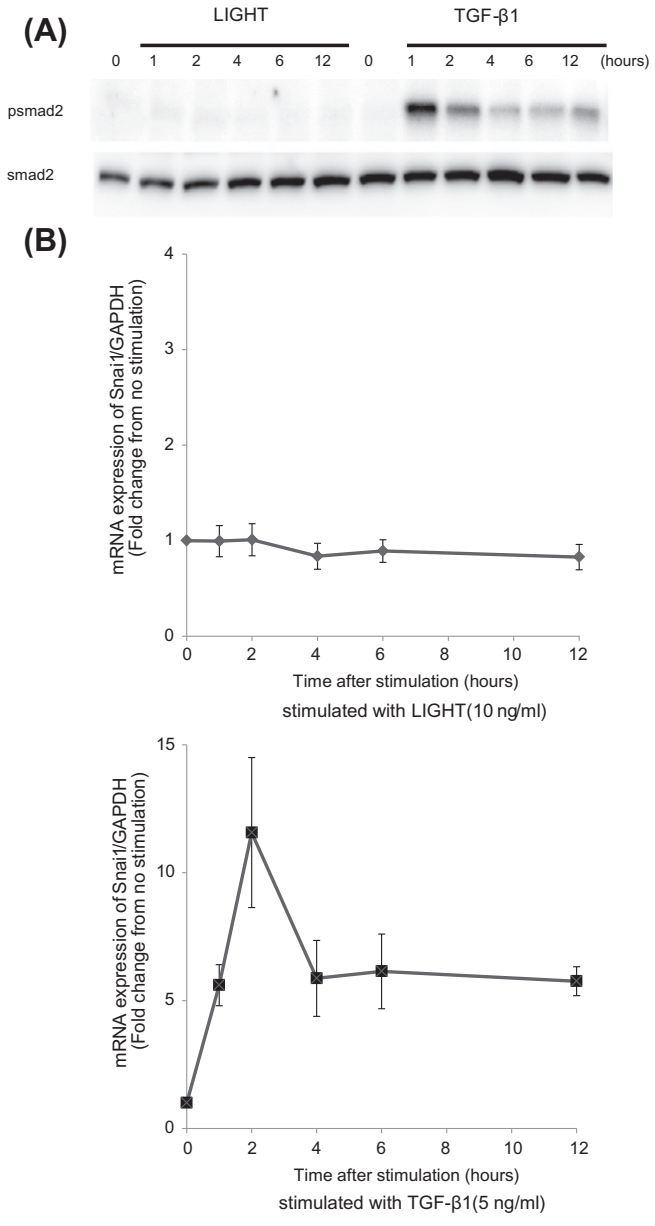

Fig. 3. LIGHT does not affect TGF-β1 production or the smad-*snai1* pathway. (A) We demonstrated time-course of effect of LIGHT (10 ng/ml) on the smad2 pathway, which is one of the major pathways associated with the TGF-β1 receptor. TGF-β1 induced phosphorylation of smad2, but LIGHT did not. (B) We showed time-course of effect of LIGHT on the *snai1* pathway, which is one of the major repressors of E-cadherin. TGF-β1 induced expression of *snai1* mRNA, but LIGHT did not.

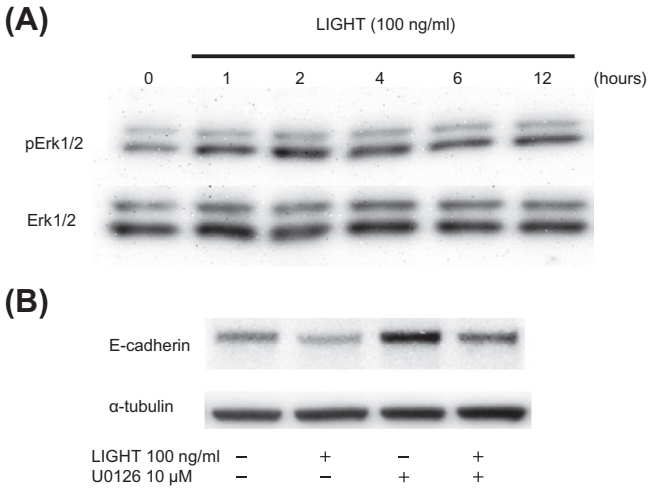

Fig. 4. Erk1/2 signaling is essential for LIGHT-induced EMT. (A) We demonstrated timecourse of effect of LIGHT on Erk1/2 phosphorylation. Erk1/2 phosphorylation was induced by LIGHT after 1 h of stimulation; the level of phosphorylation was at its peak from 2 to 4 h and then decreased by 12 h. (B) We investigated effect of Erk 1/2 inhibitor on E-cadherin expression. A549 cells were pre-treated for 1 h with U0126, a potent and specific inhibitor of Erk1/2 phosphorylation. U0126 prevented loss of E-cadherin.

Fig. 3A shows that LIGHT did not induce smad2 phosphorylation, whereas TGF-β1 did.

We then used q-RT-PCR to evaluate the expression of *snai1* mRNA, which works downstream of smad-signaling (Fig. 3B). *Snai1* mRNA expression was induced by TGF-β1 and was strongest at 2 h after stimulation, but LIGHT did not affect *snai1* mRNA expression. Therefore, LIGHT did not affect either TGF-β1 production or the smad-*snai1* pathway.

3.3.3. Erk1/2 signaling is essential for LIGHT-induced EMT

We next evaluated the effect of LIGHT on phosphorylation of Erk1/2. Fig. 4A shows that LIGHT induced Erk1/2 phosphorylation after 1 h of stumulation; the level of phosphorylation was at its peak from 2 to 4 h and then decreased by 12 h. To determine whether the Erk1/2 pathway was involved in LIGHT-induced EMT, we treated A549 cells with pharmacological inhibitors of the pathway prior to induction of EMT with LIGHT. We used U0126, a potent and specific inhibitor of Erk1/2 phosphorylation, to inhibit the Erk1/2 pathway. Fig. 4B shows that pre-treatment of A549 cells with U0126 prevented loss of E-cadherin. Taken together, these results show that Erk1/2 signaling is essential for LIGHT-induced EMT.

4. Discussion

This study demonstrated that LIGHT enhanced TGF-β1-induced EMT as well as the contractility of cells that underwent EMT. It also generated the first evidence that LIGHT itself induces EMT, without TGF-β1. LIGHT-induced EMT was shown to be an Erk1/2-dependent process, characterized by morphological transition from a typically epithelial cobblestone appearance to spindle-shaped, elongated cells expressing mesenchymal markers—vimentin, N-cadherin and α-SMA—with simultaneous loss of an epithelial protein—E-cadherin—that is an important caretaker of the epithelial phenotype and function [5].

Recently, LIGHT was reported to be involved in various inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease [8,25]. In severe asthma, which is a chronic airway inflammatory disease, LIGHT levels in the sputum of asthma

patients were negatively associated with lung function [7], suggesting that LIGHT is associated with airway remodeling. LIGHT induced airway fibrosis in severe asthma by enhancing secretion of TGF-β from macrophages and IL-13 from eosinophils [2]. Moreover, the levels of LIGHT were increased in bronchoalveolar lavage (BAL) fluid from patients with scleroderma—a cause of pulmonary fibrosis—and lung inflammation [16]. Therefore, LIGHT is an important mediator not only in inflammatory lung disease, but also in fibrotic lung disease. However, there have been few reports concerning the mechanisms of LIGHT-associated fibrosis, leading us to examine the effect of LIGHT on EMT, which is one of the mechanisms involved in tissue fibrosis. We demonstrated that LIGHT enhanced TGF-β1-induced EMT and also induced EMT by itself, in the absence of TGF-β1. Thus, LIGHT's role in lung fibrosis and the pathogenesis of fibrotic lung disease may be played out through EMT. TGF-β1 is a key mediator in the pathogenesis of pulmonary fibrosis. Enhanced TGF-β1 signaling was reported to contribute to induction of EMT in tissue fibrosis [9,10]. Moreover, inflammatory mediators, such as pro-inflammatory cytokines, reportedly enhanced TGF-β1-induced EMT [1,12,28]. That suggests that tissue inflammation might accelerate tissue fibrosis through EMT. However, there were few reports that EMT occurred without TGF-β1 [14], so we examined the mechanism of LIGHT-induced EMT without TGF-β1. First, we assumed that the mechanism of LIGHT-induced EMT involved enhanced production of TGF-β1, since TNF-α induces TGF-β1 expression [22]. However, LIGHT did not induce either TGF-β1 production or mRNA expression. This means that the mechanism of LIGHT-induced EMT is not autocrine TGF-β1 signaling. The smad pathway and Erk phosphorylation are major pathways in TGF-β1-induced EMT [27]. Here, we found that LIGHT induced Erk phosphorylation without involving the smad pathway. Inhibition of Erk1/2 by a specific inhibitor, U0126, abrogated the decrease of E-cadherin in A549 cells. These data indicate that Erk1/2 signaling is required in LIGHT-induced EMT.

A limitation of our study is that we demonstrated EMT induction in A549 cells, which is a cancer cell line, not in normal human epithelial cells. Thus, it is difficult to extrapolate our results directly to the pathogenesis of pulmonary fibrosis. However, A549 cells are widely used to study the functions of airway epithelial cells, and several studies yielded evidence of EMT in the lung by using A549 cells as a model of airway fibrosis [15,30]. Further experiments need to be carried out using normal human airway epithelial cells.

In summary, TGF-β1 suppressed E-cadherin expression and induced vimentin expression, thereby inducing EMT in A549 human alveolar epithelial cells. LIGHT enhanced the TGF-β1-induced EMT. The cells that underwent EMT acquired contractility, which was enhanced by costimulation with LIGHT and TGF-β1. Moreover, LIGHT itself induced EMT via the Erk1/2 pathway, without TGF-β1 signaling. LIGHT may contribute to the pathogenesis of airway fibrosis through enhancement of EMT.

Author contributions

Author contributions: Y.M., Y.Y., M.M, M.K., T.J., H.T, T.N., and K.T. conception and design of research; Y.M., and Y.Y. performed experiments; Y.M., and M.K. analyzed data; Y.M., Y.Y., M.M., and T.J. interpreted results of experiments; Y.M., and Y.Y. prepared figures; Y.M. Drafted manuscript; Y.M. Edited and revised manuscript; Y.M., Y.Y., and T.K. Approved final version of manuscript; Y.M., Y.Y., H.T., T.N., and T.K.

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Disclosures

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.10.097.

References

[1] J. Camara, G. Jarai, Epithelial–mesenchymal transition in primary human bronchial epithelial cells is Smad-dependent and enhanced by fibronectin and TNF-alpha, Fibrogenesis & Tissue Repair 3 (2010) 2.
[2] T.A. Doherty, P. Soroosh, N. Khorram, S. Fukuyama, P. Rosenthal, J.Y. Cho, P.S. Norris, H. Choi, S. Scheu, K. Pfeffer, B.L. Zuraw, C.F. Ware, D.H. Broide, M. Croft, The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling, Nature Medicine 17 (2011) 596–603.
[3] M. Forino, R. Torregrossa, M. Ceol, L. Murer, M. Della Vella, D. Del Prete, A. D'Angelo, F. Anglani, TGFbeta1 induces epithelial–mesenchymal transition, but not myofibroblast transdifferentiation of human kidney tubular epithelial cells in primary culture, International Journal of Experimental Pathology 87 (2006) 197–208.
[4] F. Grinnell, Fibroblasts, myofibroblasts, and wound contraction, The Journal of Cell Biology 124 (1994) 401–404.
[5] T.L. Hackett, Epithelial–mesenchymal transition in the pathophysiology of airway remodelling in asthma, Current Opinion in Allergy and Clinical Immunology 12 (2012) 53–59.
[6] W.D. Hardie, S.W. Glasser, J.S. Hagood, Emerging concepts in the pathogenesis of lung fibrosis, The American Journal of Pathology 175 (2009) 3–16.
[7] A.T. Hastie, W.C. Moore, D.A. Meyers, P.L. Vestal, H. Li, S.P. Peters, E.R. Bleecker, National Heart, Lung, and Blood Institute Severe Asthma Research Program Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes, The Journal of Allergy and Clinical Immunology 125 (2010) 1028–1036. e1013.
[8] S. Ishida, S. Yamane, T. Ochi, S. Nakano, T. Mori, T. Juji, N. Fukui, T. Itoh, R. Suzuki, LIGHT induces cell proliferation and inflammatory responses of rheumatoid arthritis synovial fibroblasts via lymphotoxin beta receptor, The Journal of Rheumatology 35 (2008) 960–968.
[9] M. Iwano, D. Plieth, T.M. Danoff, C. Xue, H. Okada, E.G. Neilson, Evidence that fibroblasts derive from epithelium during tissue fibrosis, The Journal of Clinical Investigation 110 (2002) 341–350.
[10] R. Kalluri, E.G. Neilson, Epithelial–mesenchymal transition and its implications for fibrosis, The Journal of Clinical Investigation 112 (2003) 1776–1784.
[11] R. Kalluri, R.A. Weinberg, The basics of epithelial–mesenchymal transition, The Journal of Clinical Investigation 119 (2009) 1420–1428.
[12] S. Kamitani, Y. Yamauchi, S. Kawasaki, K. Takami, H. Takizawa, T. Nagase, T. Kohyama, Simultaneous stimulation with TGF-beta1 and TNF-alpha induces epithelial–mesenchymal transition in bronchial epithelial cells, International Archives of Allergy and Immunology 155 (2011) 119–128.
[13] T.E. King Jr., M.I. Schwarz, K. Brown, J.A. Tooze, T.V. Colby, J.A. Waldron Jr., A. Flint, W. Thurlbeck, R.M. Cherniack, Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality, American Journal of Respiratory and Critical Care Medicine 164 (2001) 1025–1032.
[14] C.W. Li, W. Xia, L. Huo, S.O. Lim, Y. Wu, J.L. Hsu, C.H. Chao, H. Yamaguchi, N.K. Yang, Q. Ding, Y. Wang, Y.J. Lai, A.M. LaBaff, T.J. Wu, B.R. Lin, M.H. Yang, G.N. Hortobagyi, M.C. Hung, Epithelial–mesenchymal transition induced by TNF-alpha requires NF-kappaB-mediated transcriptional upregulation of Twist1, Cancer Research 72 (2012) 1290–1300.
[15] X. Liu, Inflammatory cytokines augments TGF-beta1-induced epithelial–mesenchymal transition in A549 cells by up-regulating TbetaR-I, Cell Motility and the Cytoskeleton 65 (2008) 935–944.
[16] I.G. Luzina, S.P. Atamas, R. Wise, F.M. Wigley, J. Choi, H.Q. Xiao, B. White, Occurrence of an activated, profibrotic pattern of gene expression in lung CD8+

T cells from scleroderma patients, Arthritis and Rheumatism 48 (2003) 2262–2274.
[17] D.N. Mauri, R. Ebner, R.I. Montgomery, K.D. Kochel, T.C. Cheung, G.L. Yu, S. Ruben, M. Murphy, R.J. Eisenberg, G.H. Cohen, P.G. Spear, C.F. Ware, LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator, Immunity 8 (1998) 21–30.
[18] W.Z. Mehal, J. Iredale, S.L. Friedman, Scraping fibrosis: expressway to the core of fibrosis, Nature Medicine 17 (2011) 552–553.
[19] A.G. Nicholson, L.G. Fulford, T.V. Colby, R.M. du Bois, D.M. Hansell, A.U. Wells, The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis, American Journal of Respiratory and Critical Care Medicine 166 (2002) 173–177.
[20] G. Raghu, K.K. Brown, W.Z. Bradford, K. Starko, P.W. Noble, D.A. Schwartz, T.E. King Jr., Idiopathic Pulmonary Fibrosis Study G, A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis, The New England Journal of Medicine 350 (2004) 125–133.
[21] H.Y. Reynolds, Lung inflammation and fibrosis: an alveolar macrophage-centered perspective from the 1970s to 1980s, American Journal of Respiratory and Critical Care Medicine 171 (2005) 98–102.
[22] D.E. Sullivan, M. Ferris, D. Pociask, A.R. Brody, Tumor necrosis factor-alpha induces transforming growth factor-beta1 expression in lung fibroblasts through the extracellular signal-regulated kinase pathway, American Journal of Respiratory Cell and Molecular Biology 32 (2005) 342–349.
[23] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial–mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.
[24] J.P. Thiery, J.P. Sleeman, Complex networks orchestrate epithelial–mesenchymal transitions, Nature Reviews Molecular Cell Biology 7 (2006) 131–142.
[25] J. Wang, R.A. Anders, Q. Wu, D. Peng, J.H. Cho, Y. Sun, R. Karaliukas, H.S. Kang, J.R. Turner, Y.X. Fu, Dysregulated LIGHT expression on T cells mediates intestinal inflammation and contributes to IgA nephropathy, The Journal of Clinical Investigation 113 (2004) 826–835.
[26] T.A. Wynn, Cellular and molecular mechanisms of fibrosis, The Journal of Pathology 214 (2008) 199–210.
[27] L. Xie, B.K. Law, A.M. Chytil, K.A. Brown, M.E. Aakre, H.L. Moses, Activation of the Erk pathway is required for TGF-beta1-induced EMT in vitro, Neoplasia 6 (2004) 603–610.
[28] Y. Yamauchi, T. Kohyama, H. Takizawa, S. Kamitani, M. Desaki, K. Takami, S. Kawasaki, J. Kato, T. Nagase, Tumor necrosis factor-alpha enhances both epithelial–mesenchymal transition and cell contraction induced in A549 human alveolar epithelial cells by transforming growth factor-beta1, Experimental Lung Research 36 (1) (2010) 12–24.
[29] S. Yang, S. Banerjee, A. de Freitas, Y.Y. Sanders, Q. Ding, S. Matalon, V.J. Thannickal, E. Abraham, G. Liu, Participation of miR-200 in pulmonary fibrosis, The American Journal of Pathology 180 (2012) 484–493.
[30] Q. Zhong, B. Zhou, D.K. Ann, P. Minoo, Y. Liu, A. Banfalvi, M.S. Krishnaveni, M. Dubourd, L. Demaio, B.C. Willis, K.J. Kim, R.M. DuBois, E.D. Crandall, M.F. Beers, Z. Borok, Role of endoplasmic reticulum stress in epithelial–mesenchymal transition of alveolar epithelial cells: effects of misfolded surfactant protein, American Journal of Respiratory Cell and Molecular Biology 45 (2011) 498–509.