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Expression of 150-kDa oxygen-regulated protein (ORP150) stimulates bleomycin-induced pulmonary fibrosis and dysfunction in mice

Ken-Ichiro Tanaka [a,b], Ayano Shirai [a], Yosuke Ito [b], Takushi Namba [b], Kayoko Tahara [a], Naoki Yamakawa [a,b], Tohru Mizushima [a,b,*]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Keio University, Tokyo 105-8512, Japan
[b] Faculty of Life Sciences, Kumamoto University, Kumamoto 862-0973, Japan

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) involves pulmonary injury associated with inflammatory responses, fibrosis and dysfunction. Myofibroblasts and transforming growth factor (TGF)-β1 play major roles in the pathogenesis of this disease. Endoplasmic reticulum (ER) stress response is induced in the lungs of IPF patients. One of ER chaperones, the 150-kDa oxygen-regulated protein (ORP150), is essential for the maintenance of cellular viability under stress conditions. In this study, we used heterozygous ORP150-deficient mice ($ORP150^{+/-}$ mice) to examine the role of ORP150 in bleomycin-induced pulmonary fibrosis. Treatment of mice with bleomycin induced the expression of ORP150 in the lung. Bleomycin-induced inflammatory responses were slightly exacerbated in $ORP150^{+/-}$ mice compared to wild-type mice. On the other hand, bleomycin-induced pulmonary fibrosis, alteration of lung mechanics and respiratory dysfunction was clearly ameliorated in the $ORP150^{+/-}$ mice. Bleomycin-induced increases in pulmonary levels of both active TGF-β1 and myofibroblasts were suppressed in $ORP150^{+/-}$ mice. These results suggest that although ORP150 is protective against bleomycin-induced lung injury, this protein could stimulate bleomycin-induced pulmonary fibrosis by increasing pulmonary levels of TGF-β1 and myofibroblasts.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive and devastating chronic lung condition with poor prognosis; the mean length of survival from the time of diagnosis ranges from 2.8 to 4.2 years. No treatment has been shown to improve the prognosis for IPF patients [1]. Recent studies have suggested that lung injury associated with inflammatory responses, transforming growth factor (TGF)-β1 and myofibroblasts play important roles in the pathogenesis of IPF [1,2].

An increase in lung myofibroblasts, an intermediate cell type between fibroblasts and smooth muscle cells, has been suggested to play an important role in the atypical fibrosis and collagen deposition that observed in IPF patients [2]. It was previously thought that the origin of myofibroblasts was solely peribronchiolar and that perivascular fibroblasts transdifferentiate to myofibroblasts in response to various stimuli, in particular TGF-β1 [3]. However, recently it was revealed that lung epithelial cells undergo epithelial–mesenchymal transition (EMT) to become myofibroblasts after treatment with TGF-β *in vitro* [4,5] and that EMT of epithelial cells is induced in the lungs of IPF patients and animals with pulmonary fibrosis (bleomycin-induced pulmonary fibrosis) [4,6,7]. These results suggest that some myofibroblasts in IPF patients are derived from the EMT of lung epithelial cells.

The endoplasmic reticulum (ER) stress response is induced by the accumulation of unfolded and misfolded proteins in the ER [8,9]. ER stress response-related proteins contain not only ER chaperones (such as glucose-regulated protein (GRP)78), that confer protection against stressors by refolding unfolded and misfolded proteins in the ER, but also C/EBP homologous transcription factor, a transcription factor with apoptosis-inducing activity [10]. Since the ER stress response is induced by pathogenic conditions such as hypoxia, inflammation and toxic chemicals, it is not surprising that recent studies have suggested that the ER stress response plays an important role in diseases such as gastric ulcer and Alzheimer's disease [11,12].

Abbreviations: BALF, bronchoalveolar lavage fluid; CHOP, C/EBP homologous protein; EMT, epithelial–mesenchymal transition; FVC, forced vital capacity; GRP, glucose-regulated protein; IPF, idiopathic pulmonary fibrosis; MPO, myeloperoxidase; ORP150, 150-kDa oxygen-regulated protein; α-SMA, α-smooth muscle actin; SP-C, surfactant protein C.

* Corresponding author at: Department of Analytical Chemistry, Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, Japan. Fax: +81 3 5400 2628.
E-mail address: mizushima-th@pha.keio.ac.jp (T. Mizushima).

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One of the ER chaperones, the 150-kDa oxygen-regulated protein (ORP150), was originally identified in cultured astrocytes exposed to hypoxia [13]. Previous studies showed that expression of ORP150 is up-regulated under various pathological conditions and this up-regulation is implicated in the progression of these diseases [14,15].

It was recently suggested that IPF also involves the ER stress response, with GRP78 showing increased expression in the lungs of IPF patients and bleomycin-administered mice [16–18]. Genetic studies have revealed that mutations in the gene encoding surfactant protein C (SP-C, a protein produced by lung epithelial cells) could lead to familial interstitial pneumonia (a familial form of IPF) [19,20]. These mutations induce the ER stress response due to the accumulation of misfolded pro-SP-C [16,21]. However, because that induction of the ER stress response was observed in IPF patients without these mutations [16,18], the mechanism and role of ER stress response in IPF patients is unknown at present. Based on the cytoprotective effect provided by ER chaperones, it was considered that they would have a negative impact on the development of IPF (i.e. protective roles against IPF); however, no direct evidence (such as genetic evidence) exists to substantiate this. On the other hand, it was recently reported that the ER stress response stimulates the myofibroblastic differentiation of fibroblasts and EMT of lung epithelial cells *in vitro* [18,22]. Therefore, it is also possible that the ER stress response has a positive influence on the development of IPF. Thus, to understand the role of ER stress response in IPF, it may prove useful in the first instance to examine artificially induced pulmonary fibrosis in transgenic mice expressing each protein related to the response.

In line with this, we compare here aspects of bleomycin-induced pulmonary fibrosis in heterozygous ORP150-deficient mice ($ORP150^{+/-}$ mice) and wild-type mice. Bleomycin-induced pulmonary inflammatory responses were slightly exacerbated, while pulmonary fibrosis and dysfunction were clearly ameliorated in $ORP150^{+/-}$ mice compared to wild-type mice. We also found that bleomycin-induced increases in the pulmonary levels of both active TGF-β1 and myofibroblasts were suppressed in $ORP150^{+/-}$ mice. These results suggest that ORP150 is stimulative for bleomycin-induced pulmonary fibrosis, though at the same time ORP150 exerts a protective action against bleomycin-induced lung damage.

2. Materials and methods

2.1. Animals

Mice heterozygous for a truncated/inactivated mutant form of ORP150 ($ORP150^{+/-}$) and their wild-type counterparts ($ORP150^{+/+}$) (6–8 weeks of age) were prepared as described previously [12]. The experiments and procedures described here were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health, and were approved by the Animal Care Committee of Keio University and Kumamoto University.

2.2. Administration of bleomycin, preparation of bronchoalveolar lavage fluid (BALF), assay for MPO activity, analysis of lung function and histological and immunohistochemical analyses

Mice were maintained under anaesthesia with chloral hydrate (500 mg/kg) and were given one intratracheal injection of bleomycin (5 mg/kg) to induce pulmonary fibrosis.

BALF was collected by cannulating the trachea and lavaging the lung with 1 ml of sterile PBS containing 50 units/ml heparin (two times). The total cell number in the BALF was counted using a haemocytometer. Cells were stained with Diff-Quik reagents and the ratios of alveolar macrophages, lymphocytes and neutrophils to total cells were determined. More than 200 cells were counted for each sample.

Myeloperoxidase (MPO) activity in the lung was measured as described previously [23].

Analysis of lung function was performed with a computer-controlled small-animal ventilator (FlexiVent; SCIREQ, Montreal, Canada) as described previously [24].

Histological and immunohistochemical analyses were done as described previously [24].

2.3. Hydroxyproline determination and ELISA for active TGF-β1

Hydroxyproline content was determined as described [25]. The active TGF-β1 content in the lung was determined by ELISA as described previously [24].

2.4. Immunoblotting analysis and real-time RT-PCR analysis

Total protein was prepared as described previously [26]. Samples were applied to polyacrylamide SDS gels and subjected to electrophoresis, after which the proteins were immunoblotted with an antibody against ORP150.

Real-time RT-PCR was performed as previously described [27]. The primers used were (name: forward primer, reverse primer): *Orp150*: 5′-gaagatgcagagcccatttc-3′, 5′-tctgctccaggacctcctaa-3′; *α-sma*: 5′-catcatgcgtctggatctgg-3′, 5′-ggacaatctcacgctcagca-3′; *E-cadherin*: 5′-tgcccagaaaatgaaaaagg-3′, 5′-gtgtatgtggcaatgcgttc-3′; *Col1a1*: 5′-ccctgtctgcttcctgtaaact-3′, 5′-catgttcggttggtcaaagata-3′; *actin*: 5′-ggacttcgagcaagagatgg-3′, 5′-agcactgtgttggcgtacag-3′.

2.5. Transfection

The siRNA for ORP150 was purchased from Qiagen. Cells were transfected with the siRNA using HiPerFect transfection reagents (Invitrogen) according to the manufacturer's instructions.

The plasmid for overexpression of ORP150 was constructed by insertion of the *Orp150* gene in pCI-neoORP150 [28] into pcDNA3.1 (Invitrogen). The transfection was carried out using Lipofectamine LTX (Invitrogen).

2.6. Statistical analysis

All values are expressed as the mean ± S.E.M. or S.D. Two-way analysis of variance followed by the Tukey test was used to evaluate differences between more than three groups. Differences were considered to be statistically significant for values of $p < 0.05$.

3. Results

3.1. Effect of down-regulation of ORP150 expression on bleomycin-induced pulmonary inflammatory responses

Pulmonary inflammation and fibrosis were induced by a once-only (at day 0) intratracheal administration of bleomycin, as described previously [25]. First, we monitored the expression of ORP150 in lung tissues by immunoblotting. As shown in Fig. 1A–D, treatment of mice with bleomycin increased the pulmonary level of ORP150 in $ORP150^{+/-}$ mice and wild-type mice, though the $ORP150^{+/-}$ mice showed lower ORP150 expression than the wild-type mice both in the presence and absence of bleomycin treatment.

The bleomycin-induced inflammatory response can be monitored in terms of the number of inflammatory cells in BALF after the administration of bleomycin [25]. As shown in Fig. 2A, the total

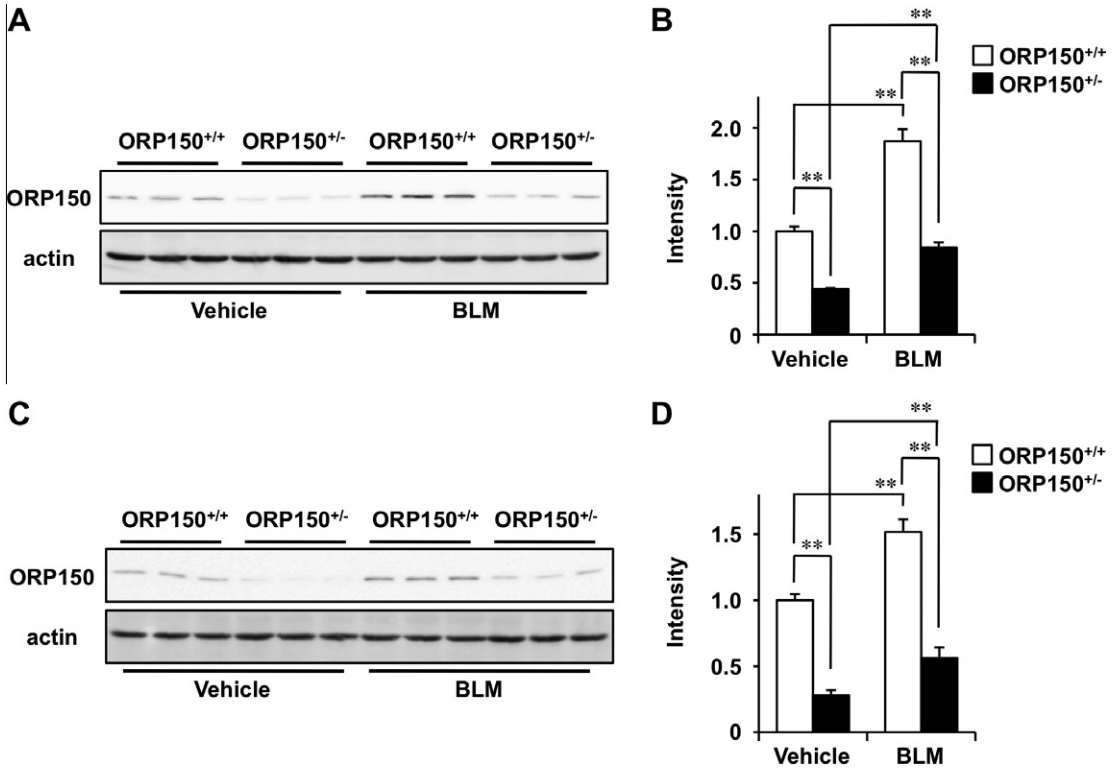

Fig. 1. Bleomycin-induced expression of ORP150 in the lung. ORP150$^{+/-}$ mice and wild-type mice (ORP150$^{+/+}$) were treated with (BLM) or without (Vehicle) bleomycin (5 mg/kg) once-only on day 0. Lungs were excised on day 3 (A and B) or 14 (C and D). Tissue homogenates were analysed by immunoblotting with antibodies against ORP150 or actin (A and C). The band intensity of ORP150 was determined and normalised to actin intensity (B and D). Values are mean ± S.E.M. ($n = 3$). $^{**}p < 0.01$. Scale bar, 50 μm.

number of inflammatory cells and individual numbers of alveolar macrophages, lymphocytes and neutrophils were all increased on day 3 after the bleomycin treatment. Compared to wild-type mice, this increase was slightly enhanced in ORP150$^{+/-}$ mice (Fig. 2A). Similar results were observed for MPO activity, an indicator of the inflammatory infiltration of leucocytes (Fig. 2B). These results suggest that ORP150 expression protects against bleomycin-induced inflammatory response.

3.2. Effect of down-regulation of ORP150 expression on bleomycin-induced pulmonary fibrosis, alteration of lung mechanics and respiratory dysfunction

Bleomycin-induced pulmonary fibrosis can be assessed by histopathological analysis and measurement of hydroxyproline levels. Haematoxylin and eosin (H & E) staining and Masson's trichrome staining of collagen revealed that bleomycin-induced collagen deposition was less apparent in ORP150$^{+/-}$ mice than in wild-type mice (Fig. 3A and B). Bleomycin also increased pulmonary hydroxyproline levels, although to a lesser degree in the ORP150$^{+/-}$ mice compared with wild-type mice (Fig. 3C). These results suggest that bleomycin-induced pulmonary fibrosis is less extensive in ORP150$^{+/-}$ mice compared to wild-type mice.

We then examined the effect of ORP150 expression on bleomycin-induced alterations of lung mechanics using a computer-controlled small-animal ventilator. Total respiratory system elastance (elastance of total lung including bronchi, bronchioles and alveoli) and tissue elastance (elastance of alveoli) were indistinguishable between heterozygous and wild-type mice in the absence of bleomycin treatment (Fig. 3D). In contrast, both types of mice showed increases in these parameters in response to bleomycin treatment, with this effect being more prominent in wild-type mice. These results suggest that the bleomycin-induced alteration of lung mechanics is ameliorated by the down-regulation of ORP150 expression.

As shown in Fig. 3E, FVC was indistinguishable between vehicle-treated ORP150$^{+/-}$ and wild-type mice, but was clearly decreased by the bleomycin treatment in wild-type mice while remaining unchanged in ORP150$^{+/-}$ mice (Fig. 3E). These results suggest that ORP150 expression exacerbates bleomycin-induced respiratory dysfunction.

To elucidate the mechanism underlying this stimulative effect of ORP150 on pulmonary fibrosis, we focused our attention on myofibroblast and TGF-β1. As shown in Fig. 4A, a bleomycin-dependent increase in the expression of α-SMA, a myofibroblast marker, was suppressed in ORP150$^{+/-}$ mice compared to wild-type mice, suggesting that the expression of ORP150 stimulates an increase in pulmonary myofibroblast number in the presence of bleomycin.

As shown in Fig. 4B, active TGF-β1 was increased by the bleomycin treatment; however the level was lower in ORP150$^{+/-}$ mice than in wild-type mice. These results suggest that ORP150 expression also stimulates a bleomycin-dependent increase in active TGF-β1 in lung tissue.

3.3. Effect of ORP150 expression on the TGF-β1-induced EMT of epithelial cells and differentiation of fibroblasts in vitro

As described in Section 1, an increase in pulmonary myofibroblasts associated with fibrosis is due to the stimulation of EMT of epithelial cells and the myofibroblastic differentiation of fibroblasts. We tested whether the expression of ORP150 affects these phenomena by examining *in vitro* the effect of siRNA for ORP150 on the TGF-β1-dependent alteration of expression of EMT-related genes in cultured human type II alveolar (A549) cells. Treatment of cells with TGF-β1 down-regulated the expression of the epithelial cell

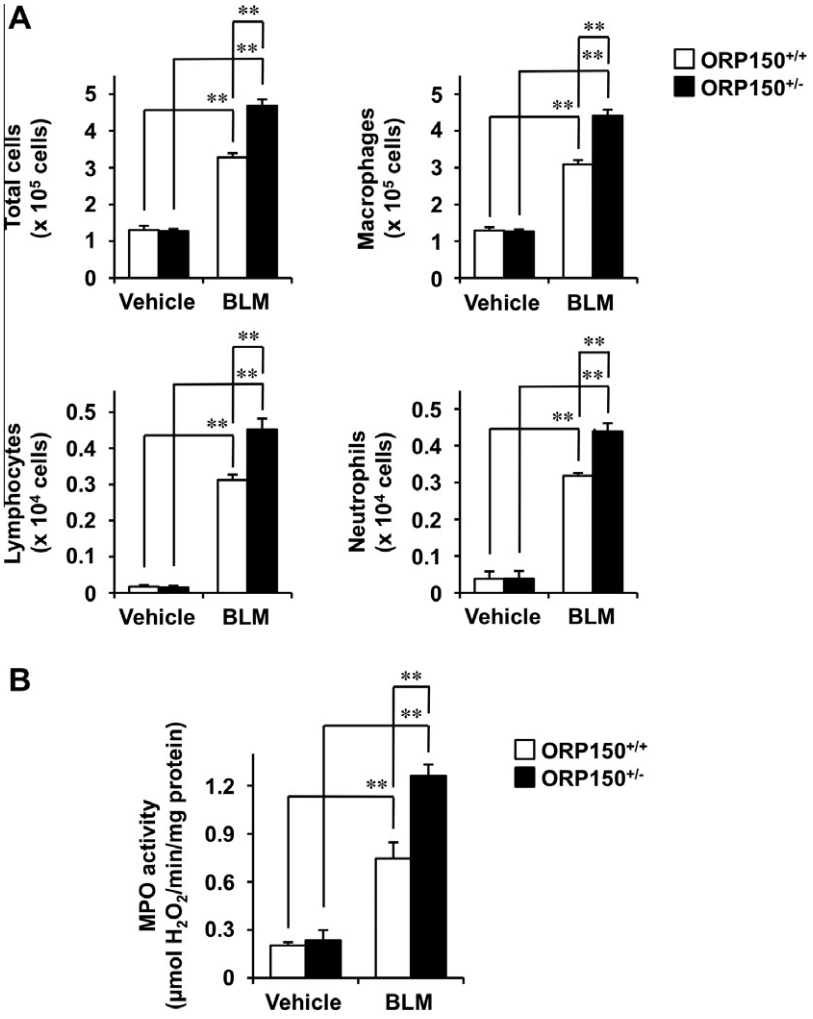

Fig. 2. Effect of down-regulation of ORP150 expression on bleomycin-induced pulmonary inflammatory responses. ORP150$^{+/-}$ mice and wild-type mice (ORP150$^{+/+}$) were treated with (BLM) or without (Vehicle) bleomycin (5 mg/kg) once-only on day 0 and lung tissue or BALF was obtained on day 3. Total cell number and individual numbers of alveolar macrophages, lymphocytes and neutrophils in BALF were counted (A). MPO activities in lung homogenates were determined (B). Values are mean ± S.E.M. $^{**}p < 0.01$.

marker *E-cadherin* mRNA (Fig. 4C), suggesting that TGF-β1 induces the EMT-like phenotype of A549 cells. Transfection of cells with siRNA for ORP150 suppressed the expression of *Orp150* mRNA but did not affect the expression of *E-cadherin* mRNA in the presence or absence of TGF-β1 (Fig. 4C). These results suggest that ORP150 expression does not affect the EMT of lung epithelial cells.

We also examined the effect of ORP150 expression on TGF-β1-dependent myofibroblastic differentiation of fibroblasts *in vitro*. Treatment of human embryonic lung fibroblasts (HFL-I cells) with TGF-β1 induced the expression of α-*sma* and *col1a1* mRNAs (Fig. 4D), suggesting that TGF-β1 activates the transition of fibroblasts to myofibroblasts. The transfection of cells with siRNA for ORP150 slightly decreased the expression of α-*sma* and *col1a1* mRNAs in the presence of TGF-β1 (Fig. 4D). We then examined the effect of overexpression of ORP150 on the myofibroblastic differentiation of fibroblasts. As shown in Fig. 4E, slightly increased the expression of α-*sma* and *col1a1* mRNAs was observed in the presence of TGF-β1. These results suggest that ORP150 expression stimulates the TGF-β1-dependent myofibroblastic differentiation of fibroblasts.

4. Discussion

Pulmonary fibrosis is triggered by pulmonary damage and inflammatory responses. In other words, pulmonary fibrosis is a result of abnormal and extended repair and remodelling of damaged lung tissues. We here showed that bleomycin-induced inflammatory responses, such as an increase in BALF leucocyte number and activation of pulmonary MPO, were stimulated in ORP150$^{+/-}$ mice, suggesting that expression of ORP150 could suppress inflammatory responses. As such, we postulated that bleomycin-induced pulmonary fibrosis would be exacerbated in ORP150$^{+/-}$ mice. However, surprisingly, this was not the case. Further to this, we found that increases in pulmonary levels of both active TGF-β1 and myofibroblasts in response to bleomycin were suppressed in ORP150$^{+/-}$ mice compared to wild-type mice. These results suggest that although expression of ORP150 is protective against bleomycin-induced inflammatory responses, it could accentuate pulmonary fibrosis by increasing pulmonary levels of TGF-β1 and myofibroblasts.

As described above, it was recently reported that induction of the ER stress response by chemicals or by expression of misfolded proteins in the ER in alveolar endothelial cells or fibroblasts causes EMT or myofibroblastic differentiation, respectively [18,22]. Therefore, it is possible that the up-regulation of ORP150 expression is responsible for the observed results. We tested this idea *in vitro* and found that the TGF-β1-dependent induction of EMT-like phenotypes in lung epithelial cells was not affected by the suppression of ORP150 expression. On the other hand, the TGF-β1-dependent activation of fibroblasts (myofibroblastic differentiation) was

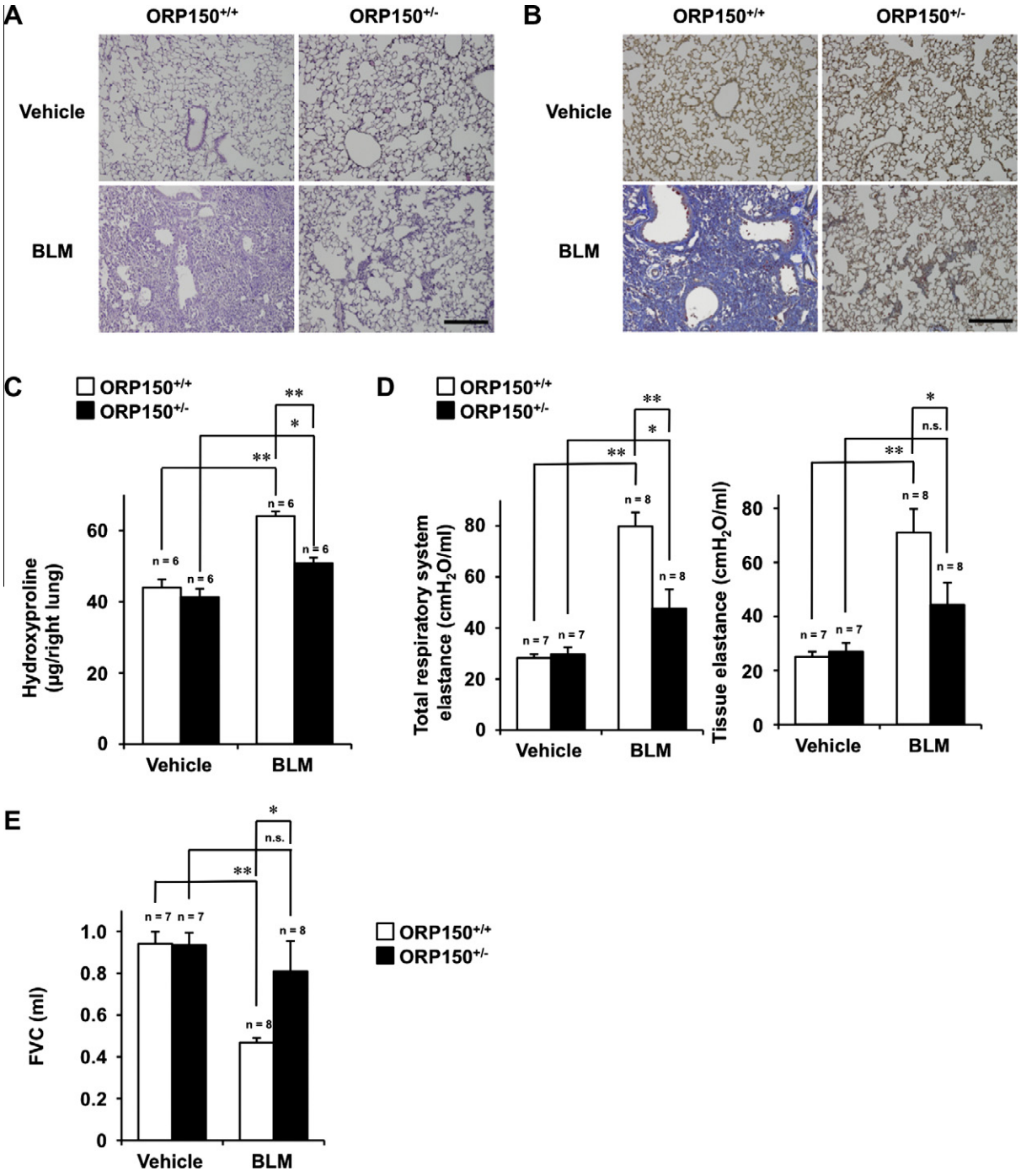

Fig. 3. Effect of down-regulation of ORP150 expression on bleomycin-induced pulmonary fibrosis, alteration of lung mechanics and respiratory dysfunction. ORP150$^{+/-}$ mice and wild-type mice (ORP150$^{+/+}$) were treated with (BLM) or without (Vehicle) bleomycin (5 mg/kg) once-only on day 0 and lung tissue removed on day 14. Sections of pulmonary tissue were subjected to H & E staining (A) or Masson's trichrome staining (B). Pulmonary hydroxyproline levels in lung homogenates were determined (C). Total respiratory system elastance (D), tissue elastance (D) and FVC (E) were determined on day 14. Values shown are mean ± S.E.M. $^{**}p < 0.01$; $^{*}p < 0.05$. Scale bar, 100 μm.

slightly suppressed or stimulated by the suppression or induction, respectively, of ORP150 expression. These results suggest that ORP150 expression stimulates the increase in lung myofibroblasts by stimulating myofibroblastic differentiation rather than by stimulating the EMT of epithelial cells.

Although assessment tools used in bleomycin-induced pulmonary fibrosis are primarily based on histological and quantitative collagen analysis, the clinical management of IPF relies on lung function analysis. We therefore used a computer-controlled small-animal ventilator to monitor the effect of bleomycin on elastance, as an increase in elastance has also been associated with human IPF [29]. We found that the bleomycin-induced alteration of lung mechanics was ameliorated in ORP150$^{+/-}$ mice compared to wild-type mice. An improvement of FVC serves as the endpoint of clinical assessments to estimate the efficacy of candidate drugs in IPF patients; to this extent we found that bleomycin-induced a decrease in FVC was ameliorated in ORP150$^{+/-}$ mice compared to wild-type mice. These results suggest that ORP150 expression stimulates not only bleomycin-induced pulmonary fibrosis but also alters lung mechanics and respiratory dysfunction.

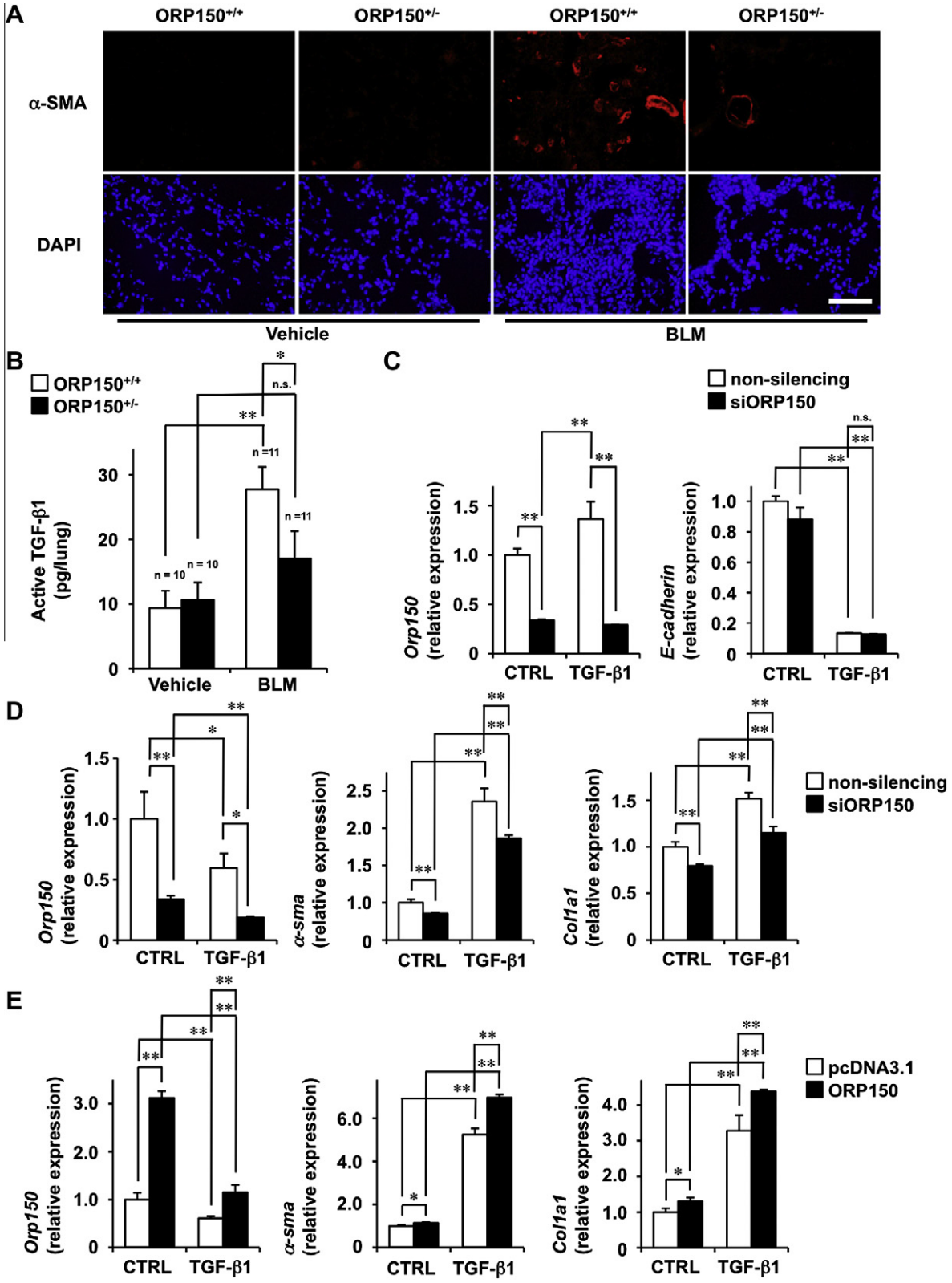

Fig. 4. Effect of down-regulation of ORP150 expression on the pulmonary levels of myofibroblasts and active TGF-β1 *in vivo* and on TGF-β1-induced EMT-like phenotypes of epithelial cells and myofibroblastic differentiation of fibroblasts *in vitro*. (A and B) ORP150$^{+/-}$ mice and wild-type mice (ORP150$^{+/+}$) were treated with (BLM) or without (Vehicle) bleomycin (5 mg/kg) once-only on day 0 and lung tissues removed on day 7 (B) or 14 (A). Sections of pulmonary tissue were subjected to immunohistochemical analysis with an antibody against α-SMA (A). Levels of active TGF-β1 in lung homogenates were measured by ELISA (B). Values shown are mean ± S.E.M. $^{**}p < 0.01$; $^{*}p < 0.05$. Scale bar, 50 μm. (C–E) A549 (C) or HFL-I (D) cells were transfected with 1.0 μg of siRNA for ORP150 (siORP150) or non-silencing siRNA and incubated for 24 h (C and D). HFL-I cells were transiently transfected with 2.5 μg of expression plasmid for ORP150 or control vector (pcDNA3.1) and cultured for 24 h (E). Cells were then incubated with 10 ng/ml TGF-β1 for 24 (D and E) or 48 h (C). Total RNA was extracted and subjected to real-time RT-PCR using a specific primer set for each gene. Values were normalised to the *actin* gene and expressed relative to the control sample. Values shown are mean ± S.E.M. ($n = 3$). $^{**}p < 0.01$; $^{*}p < 0.05$.

Taken together, the findings presented here suggest that ORP150 could serve as a possible drug target for IPF; however, this requires careful examination given that drugs that inhibit ORP150 function may also induce acute lung injury by suppressing the cytoprotective effects that ORP150 exerts against pulmonary damage.

Acknowledgments

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References

[1] American Thoracic Society, Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS), Am. J. Respir. Crit. Care Med. 161 (2000) 646–664.
[2] D. Sheppard, Transforming growth factor beta: a central modulator of pulmonary and airway inflammation and fibrosis, Proc. Am. Thorac. Soc. 3 (2006) 413–417.
[3] T. Kisseleva, D.A. Brenner, Fibrogenesis of parenchymal organs, Proc. Am. Thorac. Soc. 5 (2008) 338–342.
[4] B.C. Willis, Z. Borok, TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease, Am. J. Physiol. Lung Cell. Mol. Physiol. 293 (2007) L525–534.
[5] U. Bartram, C.P. Speer, The role of transforming growth factor beta in lung development and disease, Chest 125 (2004) 754–765.
[6] K.K. Kim, M.C. Kugler, P.J. Wolters, L. Robillard, M.G. Galvez, A.N. Brumwell, D. Sheppard, H.A. Chapman, Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix, Proc. Natl. Acad. Sci. USA 103 (2006) 13180–13185.
[7] Z. Wu, L. Yang, L. Cai, M. Zhang, X. Cheng, X. Yang, J. Xu, Detection of epithelial to mesenchymal transition in airways of a bleomycin induced pulmonary fibrosis model derived from an alpha-smooth muscle actin-Cre transgenic mouse, Respir. Res. 8 (2007) 1.
[8] R.J. Kaufman, Orchestrating the unfolded protein response in health and disease, J. Clin. Invest. 110 (2002) 1389–1398.
[9] D. Ron, Translational control in the endoplasmic reticulum stress response, J. Clin. Invest. 110 (2002) 1383–1388.
[10] H. Zinszner, M. Kuroda, X. Wang, N. Batchvarova, R.T. Lightfoot, H. Remotti, J.L. Stevens, D. Ron, CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum, Genes Dev. 12 (1998) 982–995.
[11] T. Hoshino, T. Nakaya, W. Araki, K. Suzuki, T. Suzuki, T. Mizushima, Endoplasmic reticulum chaperones inhibit the production of amyloid-beta peptides, Biochem. J. 402 (2007) 581–589.
[12] T. Namba, T. Hoshino, S. Suemasu, M. Takarada-Iemata, O. Hori, N. Nakagata, A. Yanaka, T. Mizushima, Suppression of expression of endoplasmic reticulum chaperones by *Helicobacter pylori* and its role in exacerbation of non-steroidal anti-inflammatory drug-induced gastric lesions, J. Biol. Chem. 285 (2010) 37302–37313.
[13] K. Kuwabara, M. Matsumoto, J. Ikeda, O. Hori, S. Ogawa, Y. Maeda, K. Kitagawa, N. Imuta, T. Kinoshita, D.M. Stern, H. Yanagi, T. Kamada, Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain, J. Biol. Chem. 271 (1996) 5025–5032.
[14] K. Ozawa, M. Miyazaki, M. Matsuhisa, K. Takano, Y. Nakatani, M. Hatazaki, T. Tamatani, K. Yamagata, J. Miyagawa, Y. Kitao, O. Hori, Y. Yamasaki, S. Ogawa, The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes, Diabetes 54 (2005) 657–663.
[15] T. Nakagomi, O. Kitada, K. Kuribayashi, H. Yoshikawa, K. Ozawa, S. Ogawa, T. Matsuyama, The 150-kilodalton oxygen-regulated protein ameliorates lipopolysaccharide-induced acute lung injury in mice, Am. J. Pathol. 165 (2004) 1279–1288.
[16] W.E. Lawson, P.F. Crossno, V.V. Polosukhin, J. Roldan, D.S. Cheng, K.B. Lane, T.R. Blackwell, C. Xu, C. Markin, L.B. Ware, G.G. Miller, J.E. Loyd, T.S. Blackwell, Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection, Am. J. Physiol. Lung Cell. Mol. Physiol. 294 (2008) L1119–L1126.
[17] M. Korfei, C. Ruppert, P. Mahavadi, I. Henneke, P. Markart, M. Koch, G. Lang, L. Fink, R.M. Bohle, W. Seeger, T.E. Weaver, A. Guenther, Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 178 (2008) 838–846.
[18] H.A. Baek, D.S. Kim, H.S. Park, K.Y. Jang, M.J. Kang, D.G. Lee, W.S. Moon, H.J. Chae, M.J. Chung, Involvement of endoplasmic reticulum stress in myofibroblastic differentiation of lung fibroblasts, Am. J. Respir. Cell Mol. Biol. (2011).
[19] L.M. Nogee, A.E. Dunbar 3rd, S.E. Wert, F. Askin, A. Hamvas, J.A. Whitsett, A mutation in the surfactant protein C gene associated with familial interstitial lung disease, N. Engl. J. Med. 344 (2001) 573–579.
[20] A.Q. Thomas, K. Lane, J. Phillips 3rd, M. Prince, C. Markin, M. Speer, D.A. Schwartz, R. Gaddipati, A. Marney, J. Johnson, R. Roberts, J. Haines, M. Stahlman, J.E. Loyd, Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred, Am. J. Respir. Crit. Care Med. 165 (2002) 1322–1328.
[21] S. Mulugeta, V. Nguyen, S.J. Russo, M. Muniswamy, M.F. Beers, A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation, Am. J. Respir. Cell Mol. Biol. 32 (2005) 521–530.
[22] H. Tanjore, D.S. Cheng, A.L. Degryse, D.F. Zoz, R. Abdolrasulnia, W.E. Lawson, T.S. Blackwell, Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress, J. Biol. Chem. 286 (2011) 30972–30980.
[23] M. Matsuda, T. Hoshino, Y. Yamashita, K. Tanaka, D. Maji, K. Sato, H. Adachi, G. Sobue, H. Ihn, Y. Funasaka, T. Mizushima, Prevention of UVB radiation-induced epidermal damage by expression of heat shock protein 70, J. Biol. Chem. 285 (2010) 5848–5858.
[24] K. Tanaka, K. Sato, K. Aoshiba, A. Azuma, T. Mizushima, Superiority of PC-SOD to other anti-COPD drugs for elastase-induced emphysema and alteration in lung mechanics and respiratory function in mice, Am. J. Physiol. Lung Cell. Mol. Physiol. 302 (2012) L1250–L1261.
[25] K. Tanaka, T. Ishihara, A. Azuma, S. Kudoh, M. Ebina, T. Nukiwa, Y. Sugiyama, Y. Tasaka, T. Namba, K. Sato, Y. Mizushima, T. Mizushima, Therapeutic effect of lecithinized superoxide dismutase on bleomycin-induced pulmonary fibrosis, Am. J. Physiol. Lung Cell. Mol. Physiol. 298 (2010) L348–L360.
[26] K. Tanaka, Y. Tanaka, T. Namba, A. Azuma, T. Mizushima, Heat shock protein 70 protects against bleomycin-induced pulmonary fibrosis in mice, Biochem. Pharmacol. 80 (2010) 920–931.
[27] S. Mima, S. Tsutsumi, H. Ushijima, M. Takeda, I. Fukuda, K. Yokomizo, K. Suzuki, K. Sano, T. Nakanishi, W. Tomisato, T. Tsuchiya, T. Mizushima, Induction of claudin-4 by nonsteroidal anti-inflammatory drugs and its contribution to their chemopreventive effect, Cancer Res. 65 (2005) 1868–1876.
[28] T. Namba, T. Hoshino, K. Tanaka, S. Tsutsumi, T. Ishihara, S. Mima, K. Suzuki, S. Ogawa, T. Mizushima, Up-regulation of 150-kDa oxygen-regulated protein by celecoxib in human gastric carcinoma cells, Mol. Pharmacol. 71 (2007) 860–870.
[29] K. Ask, R. Labiris, L. Farkas, A. Moeller, A. Froese, T. Farncombe, G.B. McClelland, M. Inman, J. Gauldie, M.R. Kolb, Comparison between conventional and "clinical" assessment of experimental lung fibrosis, J. Transl. Med. 6 (2008) 16.