High-Mobility Group Box 1 Mediates Epithelial-to-Mesenchymal Transition in Pulmonary Fibrosis Involving Transforming Growth Factor-β1/Smad2/3 Signaling

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ABSTRACT

Epithelial-to-mesenchymal transition (EMT) is a crucial event in the cellular origin of myofibroblasts that secrete extracellular matrix in the progression of pulmonary fibrosis (PF). High-mobility group box 1 (HMGB1) is a novel mediator of EMT. However, whether this process involves the recognized transforming growth factor-β1 (TGF-β1)/Smad signaling that also contributes to EMT in PF has not yet been elucidated. Here, we developed a model of PF induced by bleomycin (BLM) in rats and conducted several simulation experiments in A549 (human) and RLE-6TN (rat) alveolar epithelial cell (AEC) lines to unravel the role of TGF-β1/Smad2/3 signaling in HMGB1-mediated EMT. We found that the levels of serum HMGB1 and lung hydroxyproline were severely elevated after BLM administration. Moreover, the protein expression of HMGB1, TGF-β1, phospho-Smad2/3 (p-Smad2/3), and mesenchymal markers including α-smooth muscle actin, vimentin, and type I collagen were significantly increased with the reduced protein expression of an epithelial marker (E-cadherin) in the rat model by Western blot or immunohistochemical analysis. In addition, the uptake of both exogenous TGF-β1 and HMGB1 by AECs could induce EMT; meanwhile, HMGB1 dramatically enhanced TGF-β1 expression and triggered Smad2/3 phosphorylation. In contrast, TGF-β1 deficiency evidently ameliorated HMGB1-mediated EMT with reduced p-Smad2/3 in A549 cells. It provides new insights that HMGB1 release from injured lungs promotes AEC damage through induction of the EMT process, in which TGF-β1/Smad2/3 signaling is activated and contributes to PF. These results suggest that HMGB1 may constitute a therapeutic target for developing antifibrotic agents for abnormal lung remodeling.

Introduction

Pulmonary fibrosis (PF), a progressive, fatal disorder with unclear etiology, occurs predominantly in middle-aged and elderly adults and has a median survival time of <3 years from the time of diagnosis (King et al., 2011; Raghu et al., 2011). It is well known that PF is characterized by the injury of alveolar epithelial cells (AECs); abnormal activation and proliferation of extracellular matrix (ECM)–producing cells (especially myofibroblasts); and excessive deposition of ECM with subsequent scar formation, distorted lung architecture, as well as impaired respiratory function (Loomis-King et al., 2013; Li et al., 2014a,b). Despite many recent studies to clarify the molecular mechanisms and treatment strategies for PF, limited substantial therapeutic interventions have been developed to reverse established PF or even halt the chronic progression to respiratory failure. Thus, extensive efforts are still needed to elucidate the mechanisms of PF and uncover novel targets.

Initial studies on abnormal lung remodeling have focused on the role of inflammation in inciting fibroblast activation and fibrosis, while the recent paradigm suggests that AECs undergoing epithelial-to-mesenchymal transition (EMT) also play a crucial role in the progression of PF (Selman and Pardo, 2003; Kalayarasan et al., 2013). EMT is characterized by the loss of epithelial proteins, such as E-cadherin, and the acquisition of new mesenchymal markers, including α-smooth muscle actin (α-SMA), vimentin, and type I collagen (Col-I). It is a reversible process in which epithelial cells transform into cells with mesenchymal characteristics that contribute to ECM secretion in the progression of PF (Vittal et al., 2007; Kalluri and Weinberg, 2009; Mateen et al., 2013). Accumulated data support that injured AECs contribute greatly to the local activation of fibroblasts and myofibroblasts, and may also directly serve as a source of these cells via the EMT process (Kim et al., 2006; Liu et al., 2014; Vyas-Read et al., 2014).

Among the reported inducers, transforming growth factor-β1 (TGF-β1) is a main mediator of EMT and is the key
cytokine in abnormal tissue remodeling, particularly in PF (Willis et al., 2005; Chen et al., 2013). TGF-β latency binding protein 1, which facilitates the release and activation of the biologically latent form TGF-β1, is detected primarily in alveolar macrophages and epithelial cells lining honeycomb cysts in areas of advanced PF (Khalil et al., 2001). TGF-β signaling is partly dependent on combining and activating type I TGF-β receptor; then the postreceptor signal transducers Smad2 and Smad3 are phosphorylated, resulting in the formation of a stable complex with Smad4, which transfers into the nuclei to act as a transcriptional regulator and then induces AEC injury via promotion of changes in EMT markers (Sullivan et al., 2011; Chen et al., 2014). Therefore, strategies disrupting TGF-β1/Smad signaling undoubtedly have therapeutic potential in the clinical treatment of PF.

High-mobility group box 1 (HMGB1) is a transcription factor-like protein that acts as a danger signal in inflammatory diseases, tissue injury, as well as fibrotic diseases (Rowe et al., 2008; Ebina et al., 2011; Ogiku et al., 2011; Lee et al., 2013; Griffin et al., 2014; Li et al., 2014a). In lung tissues from patients with PF, HMGB1 is predominantly expressed in alveolar macrophages, infiltrating inflammatory cells, and epithelial cells (Hamada et al., 2008). Recent evidence also shows that HMGB1 is a novel mediator of EMT in mouse type II AECs and human proximal tubular epithelial cells (He et al., 2007; Lynch et al., 2010). However, whether canonical TGF-β1/Smad signaling is involved in HMGB1-mediated EMT in abnormal lung remodeling has not yet been clarified.

This study was conducted to investigate the role of TGF-β1/Smad2/3 signaling in HMGB1-mediated EMT during the development of PF. Here we determined the serum profile of HMGB1 and lung level of hydroxyproline (HYP), as well as the protein levels of HMGB1, EMT markers, TGF-β1, and phosphorylated Smad2/3 (p-Smad2/3) in the lung tissues in a rat model of bleomycin (BLM)-induced PF. Meanwhile, A549 and RLE-6TN (human and rat) AEC lines were used to explore whether the uptake of exogenous HMGB1 triggered EMT in both kinds of AECs, and whether it was involved in the regulation of TGF-β1/Smad2/3 signaling. In addition, induction of TGF-β1 deficiency was performed to determine its role in HMGB1-induced EMT in A549 cells.

**Materials and Methods**

**Animals.** Sprague-Dawley rats weighing 180–220 g were purchased from the Experimental Animal Center of Anhui Medical University (Hefei, China). The animal experimental protocol was approved by the University Animal Care and Use Committee. In compliance with the relevant guidelines, all of the animals received humane care and had free access to food and water during the study.

**Reagents and Antibodies.** Recombinant human TGF-β1 (PeproTech, Rocky Hill, NJ) and HMGB1 (ProSpec, Nessa-Ziona, Israel) were purchased. The primary antibodies described here include antibodies to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Bioworld Technology, St. Louis Park, MN), β-actin (Abcam, Cambridge, MA), TGF-β1 (Abcam), p-Smad2/3 (Santa Cruz Biotechnology, Dallas, TX), HMGB1 (Abcam), E-cadherin (Abcam), α-SMA (Abcam), vimentin (Abcam), and Col-I (Abcam). In addition, Masson’s trichrome kit (Maixin Bio, Shenzhen, China), HYP assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and HMGB1 enzyme-linked immunoassay kit (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) were used in this study.

**BLM-Induced PF.** Ninety rats were randomly divided into the normal saline group (instilled with saline) and BLM group (instilled with BLM). To establish the PF model, each vial of BLM A5 hydrochloride (8 mg/vial; Laiboten Pharmaceutical Co., Ltd., Harbin, China) was dissolved in saline with a volume of 1.6 ml just before use. The PF model was induced by intratracheal instillation of BLM (5 mg/kg) in rats. On days 7, 14, and 28, the rats were first anesthetized with 10% chloral hydrate (5 ml/kg i.p.), and then the lungs and blood were harvested. The lungs were stained with H&E and Masson’s trichrome, while the content of HYP in lung tissues and the level of HMGB1 in serum were also measured. Furthermore, the protein expression of HMGB1, TGF-β1, p-Smad2/3, E-cadherin, α-SMA, vimentin, and Col-I were observed by Western blot or immunohistochemical analysis.

**Histologic Analysis.** The left rat lung tissues were fixed in 10% formaldehyde for 48 hours, dehydrated in a graded ethanol series, and subsequently embedded in paraffin. Sequential 5-μm lung sections were placed on slides and stained with H&E and Masson’s trichrome, for morphologic analysis and locating collagen expression by using the standard protocols, respectively. The slides were investigated under a light microscope (Olympus Opticals, Tokyo, Japan) with the same magnification (200×).

**HYP and HMGB1 Level Determination.** HYP level was determined in concordance with the instruction manual of the kit. The deputy lobes (80–100 mg) of each group were taken out to detect the content of HYP, which was expressed in micrograms of HYP per milligram of wet weight. The serum level of HMGB1 (nanograms per milliliter) was measured by enzyme-linked immunosassay. The absorbance of HYP and HMGB1 was measured using an automated Multiskan MK3 microplate reader (Thermo Scientific, Waltham, MA) at wavelengths of 550 nm and 450 nm.

**Immunohistochemical Analysis.** This method had been performed in our previous study (Xu et al., 2014a). Briefly, the lung sections were prepared; then the endogenous peroxidase activity was blocked using 0.3% H2O2, while the nonspecific protein staining was blocked with 1.5% normal goat serum in Tris-buffered saline with 0.2% Tween 20. The sections were incubated in goat anti-rabbit secondary antibodies for 1 hour. Visualization was performed with diaminobenzidine followed by washing with water, and then counterstained with hematoxylin, dehydrated and transparent, sections were coverslipped with oil of cypress and dried. The images were photographed with the same microscope and magnification (400×).

**Cell Culture and Isolation.** Human pulmonary epithelial cell line A549 and rat epithelial cell line RLE-6TN were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Gibco RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Life Technologies) at 37°C in a humidified atmosphere with 5% CO2. A549 and RLE-6TN cells were seeded in six-well plates at 80% confluence and then cultured in RPMI 1640 without FBS for 6 hours before being stimulated with TGF-β1 and HMGB1 in RPMI 1640 supplemented with FBS. After stimulation for 24 and 48 hours, the cells were collected and lysed for Western blot analysis.

**Small Interfering RNA.** The TGF-β1 small interfering RNA (siRNA), stTGF-β1, was synthesized according to human-specific sequence. A549 cells were divided into four groups as follows: control group, A549 cells without treatment; stTGF-β1 group, A549 cells with stTGF-β1 treatment; HMGB1 group, A549 cells with 4 ng/ml HMGB1 stimulation; HMGB1 + stTGF-β1 group, A549 cells with siTGF-β1 treatment and 4 ng/ml HMGB1 stimulation. The stTGF-β1 treatment groups were transfected with the mix of TGF-β1 siRNA and Lipofectamine 2000 (Invitrogen/Life Technologies) in Gibco Opti-MEM serum-free medium, while other groups were treated only with Opti-MEM serum-free medium for 6 hours in six-well plates according to the manufacturer’s instructions. After 6 hours of transfection, the A549 cells were treated with or without HMGB1 (4 ng/ml) for another...
24 hours in RPMI 1640 supplemented with FBS. The protein levels of TGF-β1, p-Smad2/3, and EMT markers were analyzed by Western blot. GAPDH was used as an internal reference for relative quantification. The primer sequences of TGF-β1 siRNA were as follows: TGF-β1 forward, 5'-GACAGAACUAUGCUUGATT-3'; TGF-β1 reverse, 5'-UGAAGCAUAGUUGGUUGCTT-3', which were synthesized by GenePharma (Shanghai, China).

**Western Blot Analysis.** The right lung lobes, weighing 90–100 mg, were homogenized in ice-cold radiomunoprecipitation lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) and proteinase inhibitor phenylmethylsulfonyl fluoride (Amresco, Solon, OH) cocktail. The lysis process was performed according to the instruction manual. To observe the protein expression levels of epithelial and mesenchymal markers as well as the role of HMGB1 in TGF-β1/Smad2/3 signaling, cultured A549 cells or RLE-6TN cells were washed three times with ice-cold phosphate-buffered saline and lysed in 100 µl of lysis buffer. The lung and cell lysates were centrifuged at 12,000g for 10 minutes at 4°C. After centrifugation, the supernatant was collected. Before use, the loading buffer was mixed with the supernatant in a ratio of 1:4 and then transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). Nonspecific binding to the membrane was blocked for 2 hours at room temperature with 5% nonfat dry milk (w/v) (Shanghai Bright Dairy Co., LTD, Shanghai, China) in Tris-buffered saline/Tween 20 (BOSTER, Wuhan, China) and incubated at 4°C overnight with the primary anti-HMGB1 (1:8000 diluted, 25 kDa), anti-TGF-β1 (1:1000 diluted, 44 kDa), anti–p-Smad2/3 (1:500 diluted, 55–60 kDa), anti–E-cadherin (1:500 diluted, 97 kDa), anti–α-SMA (1:400 diluted, 42 kDa), anti–vimentin (1:5000 diluted, 57 kDa), anti–β-actin (1:5000 diluted, 43 kDa), and anti-GAPDH (1:5000 diluted, 36 kDa) antibodies. On the next day, the blots were incubated with horseradish peroxidase–conjugated anti-rabbit or anti-goat IgG antibodies (ZSGB-BIO, Beijing, China) for 1 hour at room temperature. Immunodetection was developed with enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology). All experiments were performed independently at least three times. The densitometry was performed on protein bands using ImageJ analysis software (ChemiQ 4600; Bioshine, Shanghai, China). The integral optical density analysis was performed by Image-Pro Plus version 6.0 (Media Cybernetics, Rockville, MD), while β-actin or GAPDH was used as the internal reference for relative quantification.

**Statistical Analysis.** The data are presented as mean ± S.D. for each group in the in vivo and in vitro experiments. Statistical significance was determined by either the Student’s t test for comparison between means or one-way analysis of variance with a post hoc Dunnett’s test. Statistical analyses were performed by SPSS 13.0 software (IBM, Armonk, NY). *P* < 0.05 was considered to be significant.

**Results**

**Increased Release of HMGB1 and Activation of TGF-β1/Smad2/3 Signaling in BLM-Induced PF in Rats.** In the in vivo PF model, we first observed the abnormal pathologic changes after BLM administration. As shown by H&E and Masson’s trichrome staining in the lung sections, intratracheal injection of BLM led to the destruction of normal lung architecture, the infiltration of inflammatory cells in alveoli, and the extensive distribution of collagen in cell intervals (Fig. 1, A and B). Although TGF-β1 and HMGB1 have been reported to affect the pathologic process of fibrotic diseases (Hamada et al., 2008; Hu et al., 2014; Xu et al., 2014a,b), the link between them has not yet been clarified in PF.

Previous studies have shown that TGF-β1/Smad signaling and HMGB1 take part in the process of EMT (He et al., 2007; Chen et al., 2013, 2014). However, is HMGB1 involved in the activation of TGF-β1/Smad signaling in the EMT process? To confirm the link between them in the process of EMT in abnormal lung remodeling, we examined HYP level in the lungs, as it is the major constituent of collagen (Han et al., 2006), and protein levels of HMGB1, TGF-β1, p-Smad2/3, and EMT markers (E-cadherin, vimentin, and α-SMA) in BLM-induced PF in rats. We found that the level of HYP (Fig. 1C) in lung tissues and the content of HMGB1 (Fig. 1D) in serum were markedly increased after BLM treatment. The level of lung HYP increased gradually from day 7 to day 28, while the serum HMGB1 level was highest on day 7. Furthermore, compared with the normal saline group, the protein expression of α-SMA (Fig. 2A) and Col-I (Fig. 2B) was significantly increased according to immunohistochemical analysis after BLM administration. Meanwhile, the protein levels of HMGB1, TGF-β1, and p-Smad2/3 were sharply elevated in response to BLM treatment, correlating with downregulated expression of epithelial proteins such as E-cadherin and increased protein expression of mesenchymal markers including α-SMA and vimentin by Western blot analysis (Fig. 2C). However, these changes in the lungs were more obvious on day 28. These results indicate that the increased release of HMGB1, the upregulated activity of TGF-β1/Smad2/3 signaling, as well as the changes of EMT markers may be correlated during the development of PF.

**TGF-β1- and HMGB1-Induced EMT in A549 and RLE-6TN Cells.** During the pathogenesis of PF, excessive ECM is secreted mainly by abnormal activation of mesenchymal cells, including fibroblasts and myofibroblasts, which can arise from EMT. The above findings shown in Fig. 1 and Fig. 2 prompted us to confirm the role of TGF-β1 and HMGB1 in EMT in AECs. First, A549 and RLE-6TN cells were cultured and stimulated with TGF-β1 and HMGB1 for 24 and 48 hours, respectively. We found that TGF-β1 significantly decreased the protein expression of E-cadherin (epithelial marker) and increased the protein expression of α-SMA (mesenchymal marker) in A549 and RLE-6TN cells in a certain concentration range (Fig. 3). This was consistent with previous research showing that TGF-β1 is a crucial mediator of EMT (Chen et al., 2014).

To investigate the potential role of HMGB1 in both kinds of AECs, the effects of direct application of recombinant human HMGB1 to A549 and RLE-6TN cells as well as its role in TGF-β1/Smad2/3 signaling were subsequently examined. In the present study, the uptake of exogenous HMGB1 could induce EMT in both A549 and RLE-6TN cells, as shown by the changed expression profiles of EMT markers, including the downregulation of epithelial marker E-cadherin and upregulation of mesenchymal marker α-SMA (Fig. 4). Nevertheless, we surprisingly found that the stimulation with HMGB1 could not only induce EMT but also elevate the protein expression of TGF-β1 and p-Smad2/3 in both kinds of AECs in a certain concentration range (Fig. 4). These results strongly suggest that HMGB1 indeed acts as an important mediator of EMT in AECs. In addition, our data show that HMGB1 participates in activating TGF-β1/p-Smad2/3 signaling, through which HMGB1 might promote EMT in the process of PF.
TGF-β1 Silencing Inhibited HMGB1-Induced EMT in A549 and RLE-6TN Cells. The above findings provide in vitro evidence that HMGB1-induced EMT may be involved in the activation of TGF-β1/Smad signaling. Thus, we further explored whether TGF-β1/Smad2/3 acts in the downstream signaling pathway regulated by HMGB1. Consistent with the above studies, HMGB1 triggered Smad2/3 phosphorylation, increased the protein expression of mesenchymal markers (vimentin and α-SMA), and decreased the expression of an epithelial marker (E-cadherin) (Fig. 5). On the contrary, knockdown of TGF-β1 by siRNA significantly prevented EMT, with the reduction of the increased mesenchymal markers and the elevation of the decreased epithelial marker (E-cadherin) in response to the uptake of exogenous HMGB1 by A549 cells (Fig. 5). Furthermore, phosphorylation of Smad2/3 in response to HMGB1 stimulation was strongly attenuated with the deficiency of TGF-β1 (Fig. 5), suggesting that HMGB1-mediated EMT requires the activation of TGF-β1/Smad2/3 signaling.

Discussion

PF is a complex disease with a poor prognosis and currently few effective therapies, which reflects our limited understanding of its basic mechanisms. To our current knowledge, TGF-β1/Smad signaling and HMGB1 release play a crucial role in promoting the EMT process and triggering ECM secretion and deposition (Hamada et al., 2008; Lynch et al., 2010; Chen et al., 2014; Li et al., 2014a). Nevertheless, it has not been elucidated whether HMGB1 is involved in the recognized TGF-β1/Smad signaling that contributes to EMT in PF. In this study, we provide advanced evidence that TGF-β1/Smad2/3 signaling is required for HMGB1-induced EMT, suggesting that HMGB1 may be a potential effective target for PF treatment.

In the present study, the in vivo data showed that treating rats with BLM resulted in typical PF, including the excessive ECM deposition and lung structure destruction. Moreover, the levels of lung HYP (the major constituent of collagen) and serum HMGB1, as well as the positive protein expression of...
Col-I (one of the ingredients of collagen) and α-SMA (the marker of myofibroblasts) in the lungs, were dramatically elevated after BLM administration. Meanwhile, the protein expression of TGF-β1, p-Smad2/3, E-cadherin, vimentin, and α-SMA by Western blot analysis after BLM administration. β-Actin was used as a loading control. Data are shown as mean ± S.D. and representative of at least three separate experiments. **P < 0.01 versus normal saline (NS) group.

It is widely recognized that the regulatory mechanisms involving EMT would help to determine effective therapies to halt the progression of PF. In addition, TGF-β1 silencing inhibits the profibrotic role of HMGB1 in renal fibrosis (Lynch et al., 2010). We designed the in vitro study to investigate the role of TGF-β1 and HMGB1 in EMT in A549 and RLE-6TN cells, as well as the interplay between HMGB1 and canonical TGF-β1/Smad2/3 signaling in the process of EMT. Surprisingly, we observed that both TGF-β1 and HMGB1 can induce EMT in these AECs. In addition, our study also showed that HMGB1 increased TGF-β1 expression and triggered Smad2/3 phosphorylation. Moreover, the deficiency of TGF-β1 blocked HMGB1-induced EMT with downregulated levels of p-Smad2/3 in AECs. These results support that TGF-β1/Smad2/3 signaling is required for HMGB1-mediated EMT in AECs. However, we found that siTGF-β1 could not completely block HMGB1-mediated EMT in AECs.
indicating that canonical TGF-β1/Smad signaling may be not the only signaling pathway involved in this process. Losartan is effective in attenuating PF and cigarette smoke–induced lung injury by antagonizing TGF-β1 signaling (Yao et al., 2006; Podowski et al., 2012). Recent research also shows that losartan reduces lung damage by inhibiting the induction of HMGB1 (Hagiwara et al., 2009). Thus, it may be an interesting and meaningful exploration to investigate whether losartan inhibits TGF-β1 signaling by targeting HMGB1 in lung injury, or whether it is effective in the clinic for treating PF.

Recent studies have demonstrated that HMGB1 upregulates Smad7 expression on cardiac fibroblasts and triggers Smad2 phosphorylation in hepatic stellate cells (He et al., 2013; Kao et al., 2014). Based on these results combined with those from our present study, HMGB1 may play a significant role in Smad signaling during the development of organ fibrosis. Thus, further experiments are required to understand whether the activation of HMGB1 takes part in a non-Smad signaling pathway, the mitogen-activated protein kinase signaling pathway, in tissue fibrosis (Mishra et al., 2014). Despite current data supporting the profibrotic role of HMGB1 in PF, and recent studies showing that reducing the abnormal expression of HMGB1 attenuates PF in rats (Li et al., 2015; Zhang et al., 2015), the protective role of HMGB1 inhibitors in PF remains to be further identified.

Additionally, more detailed studies of TGF-β1/Smad signaling inhibitors should be performed to confirm the interplay
between the HMGB1 signaling and the TGF-β1/Smad signaling pathway. It was found that the receptor for advanced glycation end-products (RAGE), one of the receptors of HMGB1, is involved in the process of fibrotic changes in several organs (He et al., 2007; Rowe et al., 2008; Englert et al., 2011; Zhang et al., 2015). Further studies should investigate whether HMGB1/RAGE signaling is involved in elevating canonical TGF-β1/Smad signaling in the development of PF and which factor is the effective therapeutic target in this process. It would be easier to use transgenic animals to evaluate the involvement

Fig. 4. The uptake of exogenous HMGB1 induced EMT in AECs in a certain concentration range. The protein expression of E-cadherin, α-SMA, p-Smad2/3, and TGF-β1 was observed by Western blot analysis at 24 and 48 hours after HMGB1 stimulation in human A549 cells (A) and rat RLE-6TN cells (B). β-Actin or GAPDH was used as a loading control. Data are shown as mean ± S.D. and representative of at least three separate experiments. *P < 0.05; **P < 0.01 versus control group (without HMGB1 stimulation).

Fig. 5. Gene silencing of TGF-β1 significantly inhibited HMGB1-mediated EMT and Smad2/3 phosphorylation in AECs. Human A549 cells were first treated with siTGF-β1 for 6 hours and then treated with HMGB1 (4 ng/ml) for another 24 hours. The protein expression of TGF-β1, p-Smad2/3, E-cadherin, α-SMA, and vimentin was observed by Western blot analysis. GAPDH was used as a loading control. Data are shown as mean ± S.D. and representative of at least three separate experiments. **P < 0.01 versus control group; ##P < 0.01 versus HMGB1 group.
of HMGB1 signaling during PF and to clarify whether HMGB1 is a biomarker or potential therapeutic target for PF. Our findings in the present study demonstrated first that increased HMGB1 release is accompanied by the promotion of EMT and the activation of TGF-β1/Smad2/3 signaling in BLM-induced PF. In addition, we provided the first evidence that HMGB1 could not only induce EMT in both human A549 and rat RLE-6TN AECs, but also activate TGF-β1/Smad2/3 signaling during the above process. In addition, the inhibition of TGF-β1 could evidently ameliorate HMGB1-mediated EMT and reverse the elevation of phosphorylated Smad2/3 in AECs. In conclusion, we provide the first evidence that TGF-β1/Smad2/3 signaling involves HMGB1-mediated EMT of AECs, which may result in the activation and proliferation of ECM-producing cells, leading to the abnormal accumulation of ECM in the development of PF. It provides novel evidence for further clarifying the mechanism of PF and suggests that HMGB1 may represent a superior therapeutic target for PF treatment.

Authorship Contributions

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Contributed new reagents or analytic tools: Mo, Zhao, Zhao.
Performed data analysis: L.-C. Li, Cui, Mo.
Wrote or contributed to the writing of the manuscript: L.-C. Li, Li, Gao.

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Chen T, Nie H, Gao X, Yang J, Pu J, Chen Z, Cui X, Wang Y, Wang H, and Jia G (2013) TGF-β1-mediated epithelial mesenchymal transition of AECs involves TGF-beta R-II receptor inactivation during PF and to clarify whether HMGB1 is a biomarker or potential therapeutic target for PF. Our findings in the present study demonstrated first that increased HMGB1 release is accompanied by the promotion of EMT and the activation of TGF-β1/Smad2/3 signaling in BLM-induced PF. In addition, we provided the first evidence that HMGB1 could not only induce EMT in both human A549 and rat RLE-6TN AECs, but also activate TGF-β1/Smad2/3 signaling during the above process. In addition, the inhibition of TGF-β1 could evidently ameliorate HMGB1-mediated EMT and reverse the elevation of phosphorylated Smad2/3 in AECs. In conclusion, we provide the first evidence that TGF-β1/Smad2/3 signaling involves HMGB1-mediated EMT of AECs, which may result in the activation and proliferation of ECM-producing cells, leading to the abnormal accumulation of ECM in the development of PF. It provides novel evidence for further clarifying the mechanism of PF and suggests that HMGB1 may represent a superior therapeutic target for PF treatment.

Authorship Contributions

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