Original Article

Vimentin regulation of autophagy activation in lung fibroblasts in response to lipopolysaccharide exposure in vitro

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Background: The activation and assembly of the NLRP3 inflammasome is dependent on the interaction between NLRP3 and the intermediate filament protein vimentin in an acute respiratory distress syndrome (ARDS) model. We investigated the role of vimentin in this process using human fetal lung (HFL-1) fibroblasts with vimentin transfer genes or gene knockdown and lipopolysaccharide (LPS) intervention.

Methods: HFL-1 cells [con-vector + LPS, vimentin-pCMV3 (VIM-pCMV3), con-siRNA, and vimentin siRNA (VIM-siRNA)] were treated with LPS. An oxidative stress damage assessment, apoptosis analysis, and quantification of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-10 by enzyme linked immunosorbent assay (ELISA) were performed. Immunoblotting was used to reveal the autophagy pathway.

Results: We demonstrated that in response to LPS vimentin expression was lower in the HFL-1 cells with the vimentin gene knocked down. Specifically, an increase in oxidative stress, a decrease in mitochondrial membrane potential, or an increase in calcium ion permeability resulted in an increase in the fibroblast apoptosis rate. In addition, the inflammatory response after vimentin gene knockout was upregulated, as indicated by higher levels of TNF-a, IL-1β, IL-6, and IL-10. Importantly, the mechanism of suppression of vimentin in the lung fibroblasts was caused by a decrease in autophagy, an increase in mitochondrial membrane protein, and a decrease in mitochondrial function, which may contribute to the augmented cellular injury generated during the response to LPS.

Conclusions: This study provides insights into whether vimentin may interfere with the inflammatory cascade by activating the autophagy pathway of mitochondrial lung fibroblasts in the early stage of acute lung injury (ALI).

Keywords: Vimentin; human fetal lung-1 cells (HFL-1 cells); acute respiratory distress syndrome (ARDS); inflammation; mitochondrial autophagy

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Introduction

Sepsis is a serious systemic inflammatory state that is associated with the presence of a known or suspected infection, often with rapid onset. Patients with sepsis are usually treated in the intensive care unit, administered intravenous fluids and antibiotics, and possibly mechanical ventilation to support lung function. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) often occur in sepsis. The annual incidence

of ALI/ARDS in the United States is approximately 200,000 cases, with estimated mortality rates ranging from 25% to 60% (1). Progressive ALI occurs in one-third of patients with sepsis. ALI/ARDS is an extraordinarily complex lung disorder characterized by a neutrophilic inflammatory response and is associated with increased pulmonary vascular permeability. This destruction leads to pulmonary edema, intrapulmonary hemorrhage, and severely impaired gas exchange. The pathophysiology of ALI/ARDS in sepsis is complex and multifactorial and includes proinflammatory cytokines, chemokines, and reactive oxygen species (ROS) (2,3). Despite extensive research and progress in several fields of ALI/ARDS, the appropriate therapeutic interventions are still limited, and the mortality of patients who develop septic ALI/ARDS remains unacceptably high. Although the precise mechanisms underlying the development of ALI/ARDS following sepsis remain unclear, the inflammatory response is considered a major contributor (4,5).

Vimentin, a type III intermediate filament involved in the motility and maintenance of cell shape, has also been implicated in inflammatory responses. Apart from being a cytoskeletal protein, its alternative roles in cell biology are gaining interest. Vimentin is associated with macrophage differentiation, phagocytosis and ROS production. Vimentin is expressed on activated macrophages and is secreted in response to proinflammatory stimuli (6,7). A recent study demonstrated that the activation and assembly of the NLRP3 inflammasome is dependent on the interaction between NLRP3 and the intermediate filament protein vimentin (8). In line with our search for biomarkers useful for sepsis diagnosis and prognosis, we explored protein components by proteomics in blood samples collected from patients with sepsis or septic shock and found that serum vimentin was significantly increased in these patients (9). Furthermore, we experimentally verified that vimentin can regulate the expression of apoptosis and cytokines in lymphocytes and monocytes (9,10). Therefore, we aimed to determine whether vimentin is required for inflammasome activation and lung function in an HFL-1 cell model of LPS-induced lung injury. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-5129).

Methods

Cell culture and treatment

Human fetal lung (HFL-1) fibroblasts were purchased from American Type Collection of Culture (Manassas, VA, USA) and cultured in DMEM supplemented with 10% fetal calf serum (FCS), 50 U/mL penicillin/streptomycin and 50 mg/mL fungizone. The cells were divided every 3–5 days. The cells were treated under the following eight conditions: cells transfected with control vector (con-vector), vimentin-expressing vimentin-pCMV3 vector (VIM-pCMV3), control siRNA (con-siRNA), or vimentin-specific siRNA (VIM-siRNA), and the cells of these four conditions plus lipopolysaccharide (LPS) exposure (con-vector + LPS, VIM-pCMV3 + LPS, con-siRNA + LPS, and VIM-siRNA + LPS). For LPS intervention, the cells were treated with 1 μg/mL LPS [derived from O55:B5 *Escherichia coli* (*E. coli*); Sigma, USA] and incubated for 48 hours as described in a previous publication (11,12).

Transfection of cells with siRNA and plasmids

For siRNA transfection, HFL-1 cells were plated into 60-mm tissue culture dishes (3×10^6 cells/dish) and cultured overnight. The next day, the cells were washed once with SF-DMEM without antibiotics and amphotericin B followed by treatment with 1 mL/dish Opti-MEM. Negative control siRNA (con-siRNA, Santa Cruz Biotechnology, Cat#: sc-108060) or vimentin-specific siRNA (VIM-siRNA, Santa Cruz Biotechnology, Cat#: sc-29522) were mixed with Lipofectamine 2000 (Santa Cruz Biotechnology, Cat#: sc-29528) in Opti-MEM following the manufacturer's instructions. The mixture was then added to the 60-mm dish containing cells (500 μL/dish, final concentration of siRNA was 200 nM in a 1.5 mL/dish). After 6 h of transfection, the cells were further cultured for 24 h with 10% FCS-DMEM supplemented with antibiotics and amphotericin B overnight. Cells were then used for experiments as designed.

Plated HFL-1 cells that transfected with vimentin-overexpressing plasmid into 60 mm tissue cultures (3×10^6 cells/dish) and cultured overnight. The next day, the cells were washed once with SF-DMEM without antibiotics and amphotericin B followed by the addition of 1 mL Opti-MEM. The vimentin-expressing plasmid (pCMV3-VIM, SinoBiological Inc., Beijing, China) or an untagged negative control vector (pCMV3-untagged negative control vector) was mixed with the transfection reagent (Sinofection-293, Sino Biological Inc., Beijing, China) in Opti-MEM following the manufacturer's instructions. The mixture was then added to the 60 mm dish containing the cells (500 μL/dish, with a final concentration of siRNA of 200 nM in 1.5 mL/dish). After 6 h of transfection, the cells were further cultured for 24 h with 10% FCS-DMEM

supplemented with antibiotics and amphotericin B overnight. Cells were then used for experiments as designed.

Immunofluorescence staining

The HFL-1 cells were fixed with 4% paraformaldehyde, rinsed three times in phosphate buffer saline (PBS) for 5 min each time, and then incubated with 0.1% Triton X-100 for 10 min at room temperature. After washing three times for 5 min each, the cells were blocked with 10% bovine serum for 30 min and then incubated overnight with FITC-conjugated mouse anti-human vimentin (ab128507, Abcam, Cambridge, Massachusetts) at a 1:200 dilution at 4 ℃. After three rinses in PBS, the cells were incubated with 594-conjugated Affinipure donkey anti-rabbit IgG (H + L) (Jackson Immuno Research Inc., PA, USA) at a 1:400 dilution in bovine serum albumin (BSA)-phosphate buffered saline (PBS) for 30 min at 37 ℃. After washing, the labeled samples were examined using a confocal microscope (Leica Microsystems GmbH, Germany).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using SuperScript III (Invitrogen). Real-time PCR was performed using sequence-specific primers: vimentin-F 5'-GTCTCTGGCACGTCTTGAC-3', vimentin-R: 5'-TGCTGTTCCTGAATCTGAGC-3'; GAPDH-F: 5'-GCCTTCCGTGTCCCCACTGC-3', GAPDH-R: 5'-GGCTGGTGGTCCAGGGGTCT-3'. PCR was performed using an Eppendorf 5333 Master Cycler thermocycler (Eppendorf, lot: 5333 53658) and Eppendorf Master cycle reprealplex (Eppendorf, lot no. X226488N) with an initial denaturing step at 95 ℃ for 15 sec, 40 cycles of denaturing at 95 ℃ for 30 sec and annealing at 55 ℃ for 20 sec. Vimentin gene expression, which was normalized to GAPDH expression, was analyzed by the ΔΔCt method (13).

Detection of oxidative stress damage

Detection of oxidative stress damage included measurements of ROS, changes in mitochondrial membrane potential, and calcium accumulation. ROS were measured with a dihydroethidium fluorescence probe (Catalog #KGAF019, KeyGen Biotech, Nanjing, China). Mitochondrial membrane potential and calcium accumulation were detected using a JC-1 mitochondrial membrane potential assay kit (Catlog#10009172, Cayman, Ann Arbor, MI, USA) and Fluo-4 AM assay kit (Catalog # S1060, Beyotime Biotechnology, Shanghai, China). All these detection experiments were conducted following the manufacturers' instructions. Fluorescence changes in the HFL-1 cells were detected, photographed, and counted in at least 5 random fields of each slide with a fluorescence microscope at 200× magnification (Leica, Germany).

Apoptosis and FACS analysis

Cells (2×10^5 cells/ml/well, n=3) were seeded in 24-well plates and stimulated with LPS for 6, 12 or 24 h. The cells were harvested using 0.25% trypsin-EDTA at 37 ℃. The cells were stained with an Annexin V-EGFP apoptosis detection kit (KeyGen Biotech Co., Ltd., China) following the manufacturer's instructions and analyzed with a flow cytometer (BD Accuri™ C6 Plus flow cytometer, Accuri Cytometers Inc., Cambridge, UK). The apoptosis rate for each condition was measured by flow cytometry at least 3 times.

Quantification of tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and IL-10 by ELISAs

To assess the effect of vimentin on fibroblast release of TNF-α, IL-1β, IL-6, and IL-10, HFL-1 cells were plated in monolayers (2×10^5 cells/mL, 1 mL/well of a 12-well plate) in 10% FCS-DMEM and cultured for 2 days. The different groups of cells were then treated with 1 ml/ well serum-free DMEM (SF-DMEM). After 24 h of treatment, the medium was harvested for the quantification of cytokines by enzyme linked immunosorbent assays (ELISAs) (SEA133Hu; SEA563Hu SEA079Hu; SEA056Hu, Wuhan USCN Business Co., Ltd., China) following the manufacturer's instructions.

Immunoblotting

The cells were lysed with ice-cold cell lysis buffer. The protein concentration was then determined using a butyleyano acrylate (BCA) protein assay kit (Applygen Gene Technology Corp, Beijing). Protein samples (100 μg/lane) were separated by 10% SDS-PAGE and then transferred onto nitrocellulose membranes. After blocking with 5% skimmed milk powder in TBST (T: Tris; B: Buffer; S: Solution; T: Tween), the membranes were incubated with the following primary antibodies: anti-vimentin (Abcam cat. ab128507, 1:500 dilution), anti-COXII (CST cat.4842, 1:500 dilution),

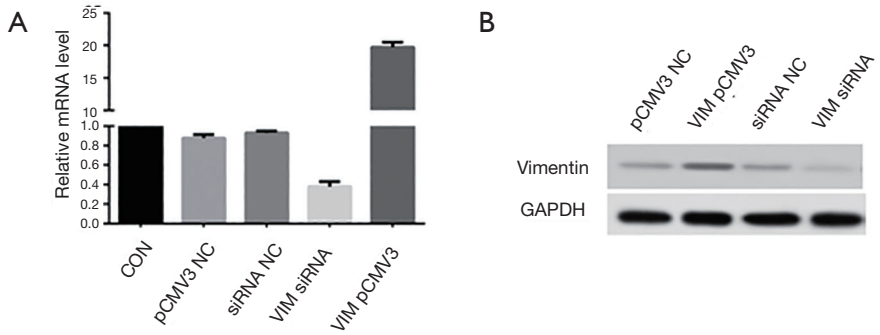

Figure 1 Vimentin expression in HFL-1 cells. HFL-1 cells were transfected with an untagged negative control vector (pCMV3-NC), a plasmid expressing vimentin (VIM-pCMV3), control siRNA (siRNA NC), and vimentin siRNA (VIM-siRNA) as described in the methods section. Representative RT-PCR (A) and immunoblotting (B) demonstrated either the overexpression or suppression of vimentin by VIM-pCMV3 or VIM-siRNA, respectively.

anti-TOM20 (BD cat.612278, 1:1,000 dilution), anti-TIM23 (BD cat.611223, 1:500 dilution), anti-Bcl-2 (CST cat.3498, 1:500 dilution), anti-caspase-3 (CST cat.9962, 1:500 dilution), and anti-P62 (MBL cat.PM045, 1:1,000 dilution), and anti-LCII (MBLcat.PM036, 1:1,000 dilution) and incubated overnight at 4 ℃. After washing, peroxidase-conjugated secondary antibodies were used. The protein bands were visualized by the electrochemiluminescence (ECL) kit. The intensities of the protein bands were analyzed by Gel-Pro 3.2 software. GAPDH (CST cat.5174, 1:1,000 dilution) was used as the internal control.

Statistical analysis

All data are expressed as the means ± SEM. Statistical comparisons of multigroup data were analyzed by ANOVA followed by Bonferroni's (two-way) or Tukey's (one-way) posttest correction using PRISM4 software.

Results

Vimentin expression in response to LPS

PCR and western blotting were performed to demonstrate vimentin gene and protein expression in HFL-1 cells after transfection with the pCMV3 vector and siRNA (*Figure 1*). Both the relative mRNA (relative mRNA level) and protein level (protein ratio *vs*. control) assays revealed that vimentin was significantly increased in the cells transfected with the VIM-pCMV3 vector but was decreased in the cells transfected with VIM-siRNA (con *vs*. VIM-pCMV3 vector *vs*. VIM-siRNA, P<0.05). These results demonstrated that successful overexpression or suppression of vimentin in the HFL-1 cells was achieved.

As shown in *Figure 2*, LPS stimulated vimentin expression in the HFL-1 cells, as evidenced by a more intense immunofluorescence than that of the control group (con *vs*. LPS group, P<0.05). Vimentin expression was further augmented by LPS in the cells transfected with the VIM-pCMV3 vector (LPS group *vs*. LPS + VIM-pCMV3 vector group, P<0.001), while it was attenuated in the cells transfected with VIM-siRNA even in the presence of LPS (LPS group *vs*. LPS + VIM-siRNA group, P<0.001). However, there were no differences between the control group, VIM-pCMV3 vector control group, and VIM-siRNA control group (P>0.05).

Oxidative stress and its damage

Measures of ROS, mitochondrial membrane potential, calcium accumulation, and apoptosis were used here to evaluate the oxidative stress damage in the HFL-1 cells following LPS exposure. As shown in *Figure 3A*, ROS was significantly increased in response to LPS (con *vs*. LPS, P<0.05, *Figure 3A*, *1* and *2*), which was further increased in the cells transfected with VIM-siRNA (LPS alone *vs*. LPS + VIM-siRNA, P<0.05, *Figure 3A*, *1* and *2*) but significantly reduced in the cells transfected VIM-pCMV3 (LPS alone *vs*. LPS + VIM-pCMV3, P<0.05, *Figure 3A*, *1* and *2*). The mitochondrial membrane potential was significantly decreased in response to LPS (con *vs*. LPS, P<0.05, *Figure 3B*, *1* and *2*), which was further decreased in the cells transfected with VIM-siRNA (LPS alone *vs*. LPS + VIM-

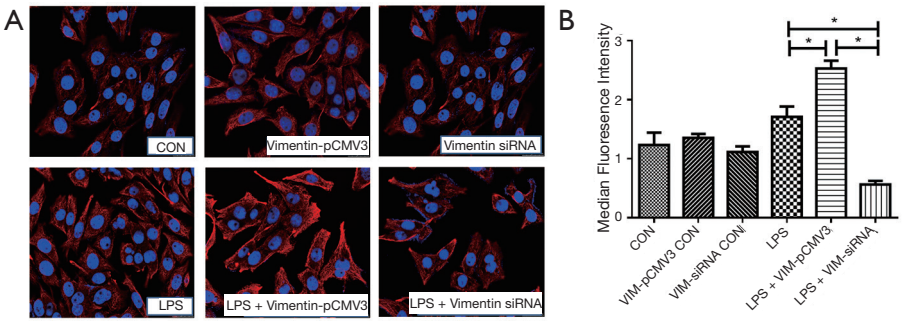

Figure 2 Vimentin expression (A) and fluorescence intensity (B) in the HFL-1 cells as visualized by immunoconfocal microscopy under a water immersion lens (400×). Red staining by the vimentin antibody reveals sites where vimentin is located. Blue spots show DAPI-stained cell nuclei. *P<0.05.

siRNA, P<0.05, *Figure 3B*, *1* and *2*) but significantly blocked in the cells transfected with VIM-pCMV3 (LPS alone *vs*. LPS + VIM-pCMV3, P<0.05, *Figure 3B*, *1* and *2*). Similarly, the intracellular calcium concentration and apoptosis rate were significantly increased for the cells treated with LPS (calcium concentration: con *vs*. LPS, P<0.05, *Figure 3C*, *1* and *2*; apoptosis: con *vs*. LPS, P<0.05, *Figure 3D*, *1* and *2*), which were further increased in the cells transfected with VIM-siRNA (calcium concentration: LPS *vs*. LPS + VIM-siRNA, P<0.05, *Figure 3C*, *1* and *2*; apoptosis: LPS *vs*. LPS + VIM-siRNA, P<0.05, *Figure 3D*, *1* and *2*) but significantly decreased in the cells transfected with VIM-pCMV3 (calcium concentration: LPS *vs*. LPS + VIM-pCMV3, P<0.05, *Figure 3C*, *1* and *2*; apoptosis: LPS *vs*. LPS + VIM-pCMV3, P<0.05, *Figure 3D*, *1* and *2*). There were no significant differences between the three groups in terms of ROS, JC-1, or calcium concentration (all P>0.05). However, there were significant differences in the calcium concentration and apoptosis rate for the control and VIM-pCMV3/VIM-siRNA groups (all P<0.05).

Release of inflammatory cytokines

TNF-α, IL-1β, IL-6, and IL-10 were measured to assess the inflammatory response. As shown in *Figure 4*, the levels of TNF-α, IL-1β, IL-6, and IL-10 were significantly increased in the LPS group compared with those in the control group (all P<0.05). In the presence of LPS, cells transfected with VIM-siRNA exhibited increased release of these cytokines (all P<0.05), while cells transfected with VIM-pCMV3 had decreased release of these cytokines (all P<0.05) in comparison to the control cells. There were no significant differences between the three groups in terms of TNF-α, IL-1β, IL-6, and IL-10 (all P>0.05).

Effect on mitochondrial membrane proteins and proteins associated with apoptosis or autophagy

As shown in *Figure 5*, the expression of mitochondrial membrane proteins (COXII, TOM20, and TIM 23) was significantly increased in the control cells in the presence of LPS (all P<0.05). The LPS + VIM-pCMV3 vector had significantly decreased the levels of the mitochondrial membrane protein COXII compared with the level in the cells exposed to LPS (*Figure 5*, P<0.05). Suppression of vimentin by siRNA plus LPS (LPS + VIM-siRNA) resulted in a further increase in these mitochondrial membrane proteins. There were no significant differences between the con, VIM-pCMV3, and VIM-siRNA groups (all P>0.05).

Regarding the expression of apoptosis-associated proteins, Bcl-2 expression was increased in the cells transfected with VIM-pCMV3 plus LPS compared to that in cells exposed only to LPS (*Figure 5A*,*E*, P<0.05). However, it was decreased in the cells transfected with VIM-siRNA plus LPS compared to those exposed only to LPS (*Figure 5A*,*E*, P<0.05). In contrast, caspase-3 was decreased in the cells transfected with VIM-pCMV3 plus LPS (*Figure 5A*,*F*, P< 0.05) but increased in the cells transfected with VIM-siRNA (*Figure 5A*,*F*, P<0.05). There were no significant differences between the con, VIM-pCMV3, and VIM-siRNA groups (all P>0.05).

Similarly, the expression of the autophagy-associated proteins P62 and LC3II was also altered in the cells. P62 was significantly decreased in the cells transfected with VIM-pCMV3 plus LPS compared with that in the cells exposed to LPS (*Figure 5A*,*G*, P<0.05). In contrast, the expression of LC3II was significantly increased in the cells transfected with VIM-pCMV3 plus LPS compared with

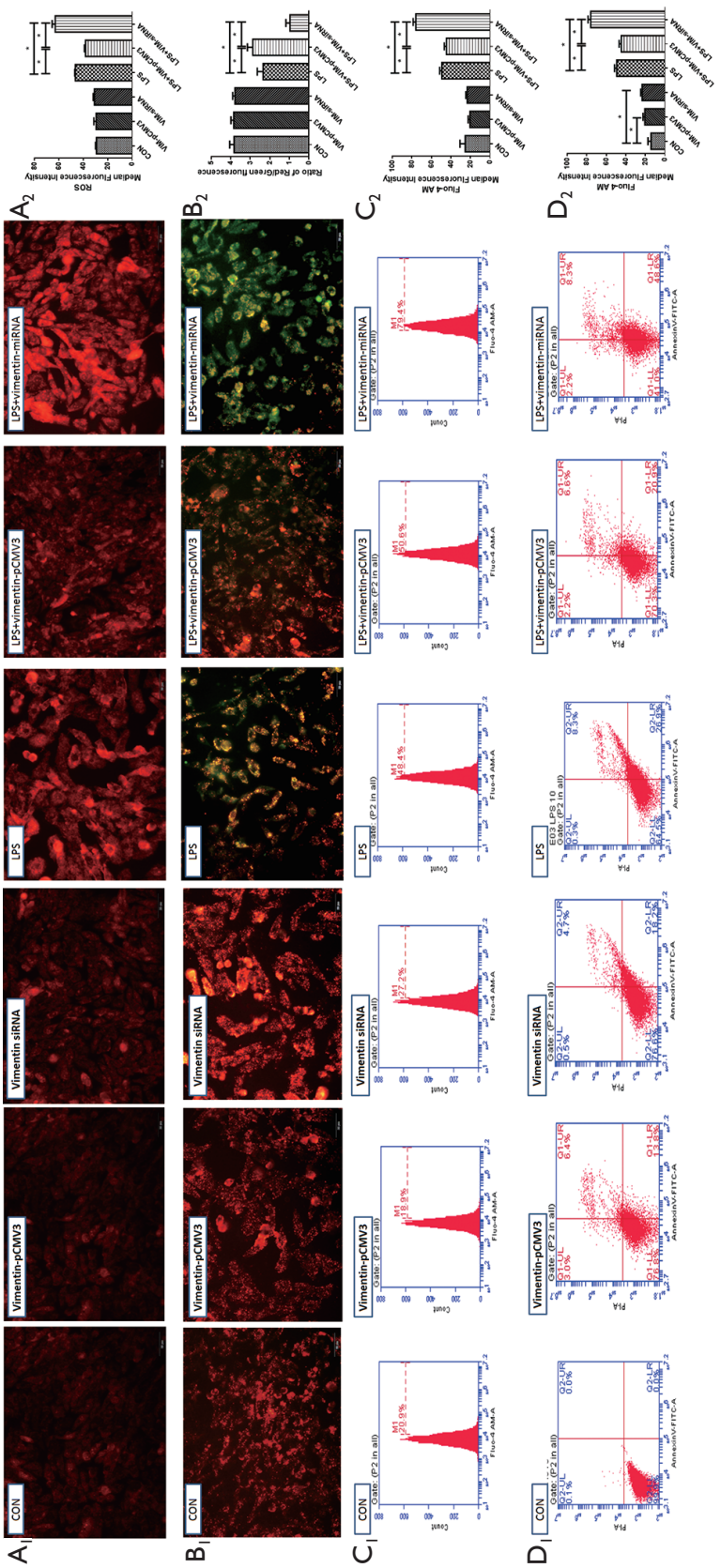

Figure 3 Oxidative stress and its damage in the HFL-1 cells in response to lipopolysaccharide (LPS). HFL-1 cells were transfected with vimentin-specific siRNA or the plasmid expressing vimentin as described in the methods. The cells were then cultured for 48 h in the presence or absence of LPS (1 μg/mL). (A) Representative images of dihydroethidium used to detect reactive oxygen species (ROS) and their median fluorescence intensity in each group. (B) Fluorescence changes in the mitochondrial membrane potential and ratio of red/green fluorescence in each group. (C) Effect on calcium accumulation in the cells measured by Fluo-4 AM. (D) Effect on apoptosis as assessed by Annexin-V assay. *P<0.05. The data presented are from one representative assessment, which was repeated at least 3 times with similar results.

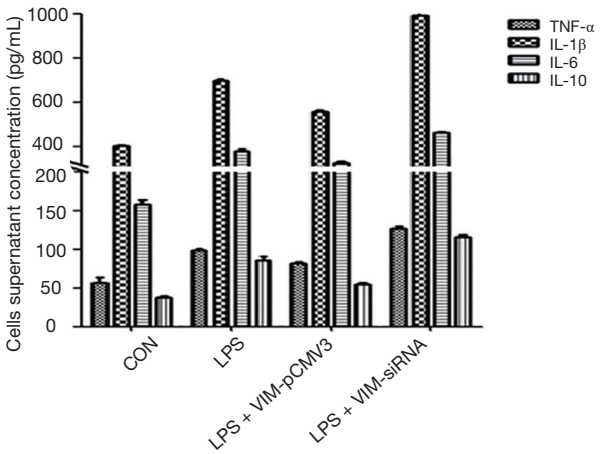

Figure 4 Release of cytokines by the HFL-1 cells in response to LPS. HFL-1 cells were transfected with vimentin-specific siRNA or a plasmid expressing vimentin as described in the method. The cells were then cultured in serum-free DMEM in the presence or absence of LPS (1 μg/mL) for 48 h. Supernatants were harvested and used for quantification of the cytokines by ELISAs as described in the methods section. Vertical axes: cytokine amount expressed as pg/mL/10^5 cells; horizontal axes: HFL-1 control cells, cells treated with LPS, cells treated with LPS after transfection of the plasmid expressing vimentin (VIM-pCMV3) or vimentin-specific siRNA (VIM-siRNA). The data presented are from one representative assessment, which was repeated 3 times with similar results. All data show statistically significant expression differences between each group (P<0.05).

that in the cells exposed only to LPS (*Figure 5A,G*, P<0.05). There were no significant differences between the con, VIM-pCMV3, and VIM-siRNA groups (all P>0.05).

Discussion

In this study, we reported that following the suppression of vimentin in human lung fibroblasts, cellular injury was aggravated by LPS intervention. Specifically, an increase in oxidative stress, a decrease in mitochondrial membrane potential, and an increase in calcium ion permeability resulted in an increase in the level of fibroblast apoptosis. Suppression of vimentin in lung fibroblasts also caused a decrease in autophagy, an increase in mitochondrial membrane protein, and a decrease in mitochondrial function, which might have contributed to the augmented cellular injury in response to LPS exposure (*Figure 6*).

When the lung is exposed to endogenous or exogenous factors, the defense mechanism is initiated. Alveolar macrophages are first activated, and a large number of inflammatory cells, especially neutrophils, are infiltrated, resulting in a large amount of ROS. Cells release inflammatory mediators, causing more inflammatory cells to aggregate, and inflammatory mediators can cause alveolar macrophages, alveolar epithelial cells, endothelial cells, and interstitial cells to produce more ROS, leading to "oxidative bursts" that cause lung tissue damage (2). Our previous study found that vimentin partially blocked the LPS-induced inflammatory response and the apoptosis of lymphocytes and monocytes, but the role of vimentin in interstitial lung cells and tissue damage was unknown. In this study, we observed that in the absence of vimentin, LPS increased the level of ROS in the lung fibroblasts, resulting in a decrease in cell membrane potential, a large influx of calcium ions, and an increased apoptosis rate. These findings suggested that ROS play important roles in mediating damage to tissue consisting of stromal cells, and vimentin may protect fibroblasts and reduce tissue damage in the early stage of ALI/ARDS.

Abnormal inflammatory responses play important roles in the development of ALI/ARDS, especially LPS-induced ALI/ARDS (3). The inflammatory response in ALI/ARDS is directly related to primary infections such as pneumonia and is also a pulmonary manifestation of systemic inflammation. Inflammatory responses and cytokine balance, as well as biological inhibitors and related molecules in the milieu of ALI, may be a key factor in the damage and repair of lung tissue (14). Inflammatory mediators can be secreted by activated cells recruited into the alveolar space under the inflammatory cascade or by dead cells (14).

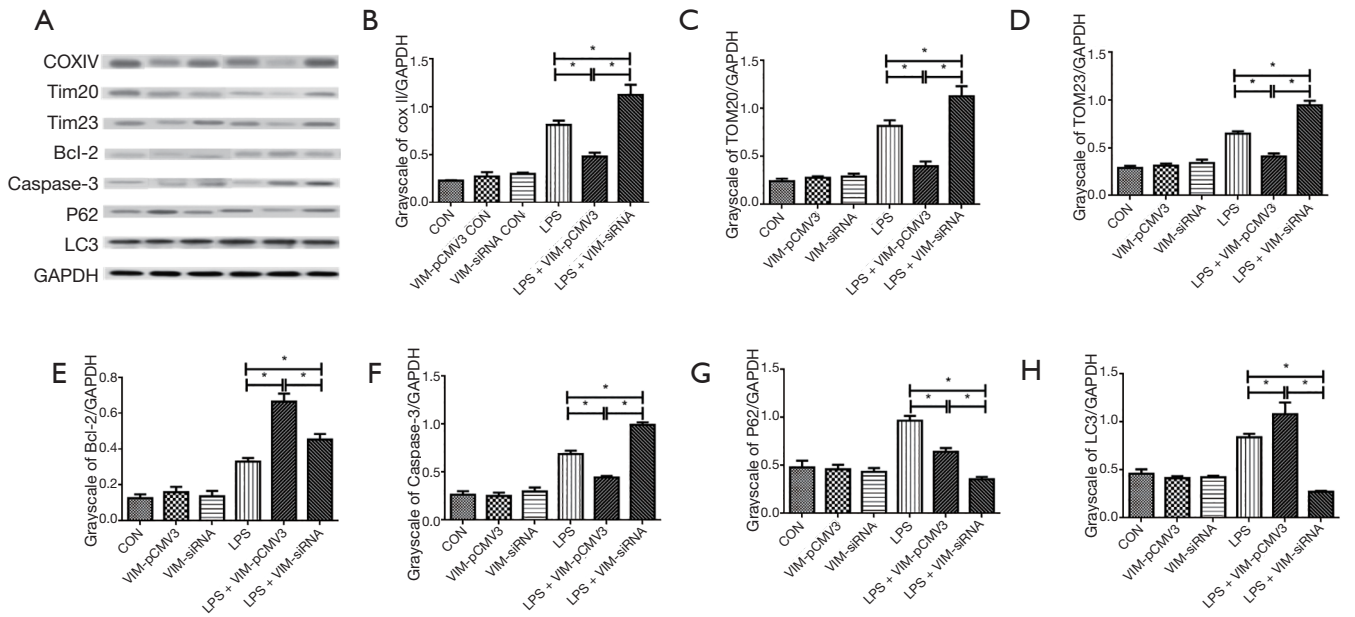

Figure 5 Expression of mitochondrial membrane proteins and apoptosis- or autophagy-associated proteins in the HFL-1 cells lacking vimentin or overexpressing vimentin. The HFL-1 cells were transfected with vimentin-specific siRNA or a plasmid expressing vimentin as described in the methods section. The cells were then cultured in serum-free DMEM in the presence or absence of LPS (1 μg/mL) for 24 h. Total cell lysates were subjected to immunoblotting for COX II, TOM20, TIM 23, caspase-3, Bcl-2, and P62, with GAPDH as the loading control, as described in the methods section. (A) Representative immunoblotting. (B) Semiquantitative comparison of COX II. (C) Semiquantification of TOM20 expression. (D) Semiquantification of TIM23 expression. (E) Semiquantification of Bcl-2 expression. (F) Semiquantification of caspase-3 expression. (G) Semiquantification of P62 expression. (H) Semiquantification of LC3II expression. Vertical axes: grayscale of targeted proteins *vs*. GAPDH. Horizontal axes: HFL-1 control cells, cells treated with LPS, cells transfected with vimentin-specific siRNA (VIM-siRNA) or vimentin-expressing plasmid (VIM-pCMV3) plus LPS. Data are presented with significance denoted by *P<0.05.

Interleukin (IL)-1β and TNF-α are the two most important proinflammatory cytokines in the early stage of ALI/ARDS, and both can promote pulmonary edema by activating endothelial cells and inducing ROS production, promoting inflammatory cell migration, and forming lung fibrosis. IL-6 and IL-10 constitute a pair of proinflammatory and anti-inflammatory cytokines, respectively. The imbalance of proinflammatory and anti-inflammatory factors may have particular importance in the regulation of lung tissue damage and repair. Our study found that IL-1β, TNF-α, and IL-6 were increased while IL-10 was decreased in the HFL-1 cells following vimentin suppression by siRNA and LPS exposure, suggesting that vimentin may be involved in the secretion of inflammatory mediators and cytokines in response to exposure to endotoxins.

Studies have shown that Nrf2 activation can regulate oxidative stress, xenobiotic metabolism and excretion, inflammation, apoptosis, autophagy, and cellular bioenergetics. Autophagy may have a protective role against cell damage (15). Mitochondria are the main organelles involved for the degradation of endogenous ROS in the cell through respiratory chain reactions. When mitochondrial autophagy is lost, intracellular ROS production increases, which causes intracellular damage (16). This study found that suppression of vimentin in lung fibroblasts led to a decrease in mitochondrial membrane protein and mitochondrial function in response to LPS, which might have been due to a decrease in autophagy function that resulted in a large accumulation of ROS and an increase in the apoptosis rate. Unexpectedly, we found that overexpression of vimentin by transfecting VIM-pCMV3 in lung fibroblasts also resulted in the decreased expression of the mitochondrial membrane protein. This outcome might have been due to mitophagy also influencing mitochondrial function. Nevertheless, the findings of the current study indicated that vimentin may play an important role in mediating the mechanism of lung

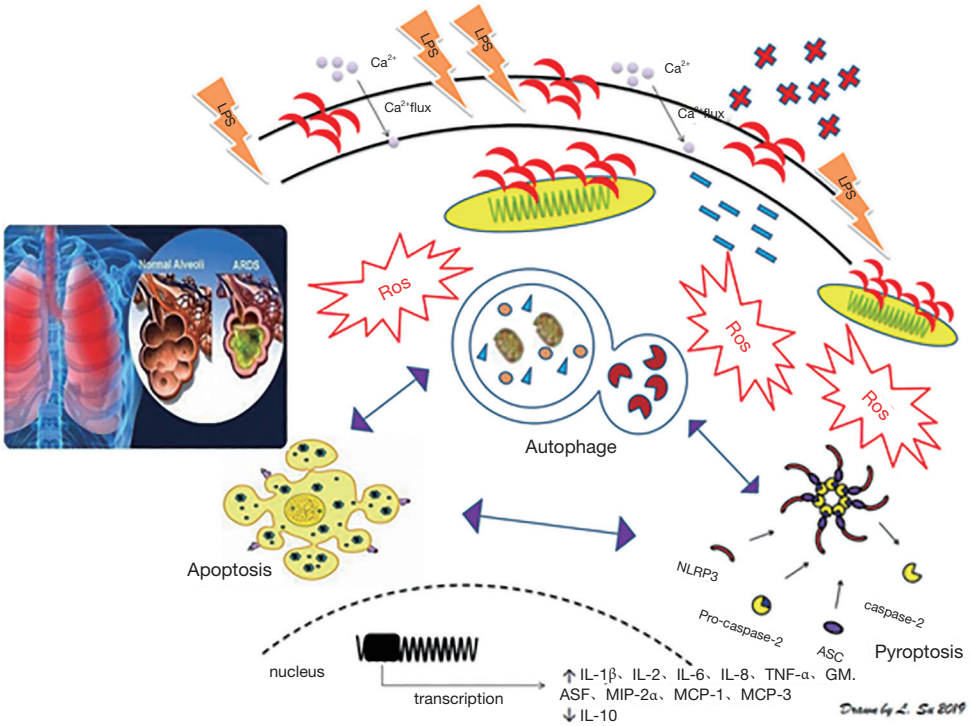

Figure 6 Vimentin regulation of the activation of autophagy in lung fibroblasts in response to LPS exposure based on an average of 3 separate experiments.

fibroblast apoptosis and autophagy in ALI/ARDS.

Oxidative stress and the inflammatory response are important components of the pathogenesis of ALI/ARDS (17,18). The basis of ARDS pathogenesis is an excessive, uncontrolled, and amplified inflammatory response cascade. During the inflammatory response, parenchymal cells and interstitial cells of the lung can produce excessive ROS through respiratory bursts to cause oxidative stress and aggravate tissue damage. Vimentin can regulate the progression of the response to oxidative stress and inflammatory responses in HFL-1 cells the early stage of ALI/ARDS though the mitochondrial protection conferred by autophagy. However, the role of vimentin in the pulmonary fibrosis period or repair process may not be the same as it is in the early stage of ALI/ARDS.

Suppression of vimentin in lung fibroblasts resulted in increased mitochondrial oxidative stress and inflammatory responses but reduced autophagy function in the cells treated with LPS. In contrast, overexpression of vimentin partially protected these lung fibroblasts from oxidative stress-induced damage and autophagy dysfunction. The findings of the current study suggested that vimentin may interfere with the inflammatory cascade by activating the mitochondrial autophagy pathway in lung fibroblasts in the early stage of ALI.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-5129). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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