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Midkine ameliorates LPS−induced apoptosis of airway smooth muscle cells *via* the Notch2 pathway

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ABSTRACT

Objective: To evaluate the effect of midkine on lipopolysaccharide (LPS)-induced airway smooth muscle cells (ASMCs).

Methods: LPS-stimulated acute lung injury model was used to analyze the effect of midkine on ASMCs *in vitro*. Recombinant midkine and midkine siRNA were used to investigate the role of Notch2 signaling pathway. Cell proliferation was assessed using Cell Counting Kit-8 assay. Additionally, apoptosis was measured by flow cytometry and protein and mRNA expression of midkine and Notch2 was assessed by Western blotting and qPCR, respectively. Immunofluorescence analysis was also conducted.

Results: LPS increased the mRNA and protein expression of midkine and Notch2. Midkine silencing reduced LPS-induced midkine and Notch2 expression. In addition, midkine silencing further reduced the viability and increased apoptosis of ASMCs induced by LPS, which was attenuated by recombinant midkine.

Conclusions: The midkine/Notch2 signaling pathway plays a regulatory role in ASMC proliferation and apoptosis in airway inflammation.

KEYWORDS: Acute lung injury; Airway smooth muscle cells; Midkine; Notch2; Lipopolysaccharide

1. Introduction

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is characterized by acute and progressive respiratory failure caused by various factors[1]. ARDS may occur at the late stage of several diseases. The occurrence and development of ALI/ARDS

Significance

Midkine participates in airway inflammation by attracting inflammatory cells, and has the effects of promoting cell proliferation and anti-apoptosis. We find that midkine promotes the proliferation of airway smooth muscle cells and reduces cell apoptosis *via* Notch2 signaling in lipopolysaccharide-induced acute lung injury model. This study may help to clarify the mechanism of airway injury in the process of acute lung injury.

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are related to microbial infections and other factors, including severe trauma, non-cardiogenic shock, poisoning, long-term cardiopulmonary resuscitation, surgery, and acute pancreatitis[2].

Midkine (MK) is a heparin-binding growth factor that promotes the chemotaxis of inflammatory cells, the release of cytokines, and inflammation[3,4]. MK activates Notch signaling[5–7]. In addition, it induces the structural recombination of actin by binding to the Notch-2 receptor, resulting in the phosphorylation and activation of STAT3, promoting cell growth and differentiation. MK can activate multiple pathways by inducing changes in the Notch-2 receptor and stimulating the expression of Hes1 and NF-κB[8]. MK is highly expressed in cystic pulmonary fibrosis and ARDS[9,10]. However, the mechanisms underlying MK actions are incompletely understood.

Airway smooth muscle cells (ASMCs) have contractile activity and produce inflammatory cytokines and growth factors, and these cells are regulated by extracellular matrix components[11,12]. The abnormal proliferation and hypertrophy of ASMCs can lead to airway wall thickening and, ultimately, airway remodeling[13]. However, it is unclear whether the MK-Notch pathway participates in ASMC proliferation. MK may inhibit apoptosis and promote ASMC proliferation through the Notch pathway, and these effects can be reversed by MK inhibition. Thus, this study aimed to evaluate the effect of MK on rat ASMCs *in vitro*.

2. Materials and methods

2.1. Cells and chemicals

Rat ASMCs (Guangzhou Gennio Biological Technology Co., Ltd China), Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA), lipopolysaccharide (LPS) (Biosharp, China), recombinant rat MK (rMK) (Beyotime Biotechnology, China), LY411575 (Selleck, USA), MK siRNA (Ribobio, China), CCK-8 kit (Dojindo Laboratories, Japan), qPCR SYBR® Green Master Mix and Liposomal Transfection Reagent (Yeasen, China), as well as FITC-coupled annexin-V apoptosis detection kit (BD Biosciences, USA) were used in the study. All other chemicals used were of analytical grade.

2.2. Cell culture and transfection

ASMCs were cultured in DMEM containing 10% fetal calf serum and incubated in a humidified incubator with 5% CO_2 at 37℃, and the medium was changed every 2 d for subcultivation.

MK siRNA transfection was done according to the manufacturer's protocol. In brief, ASMCs were seeded at 70%-80% confluence before transfection. For 96-well plates, 5 pmol of MK-siRNA and 0.25 μL of Hieff TransTM liposomal transfection reagent were diluted in 25 μL of DMEM (fetal and antibiotics free) respectively and mixed. The mixture was added to the cells after a 20-min incubation at room temperature, and then changed to the medium containing 10% fetal after 4 h. The nucleotide sequence of MK siRNA is 5'-CAAAGGCCAAAAGCCAAGAAA-3', 5'-GAAGAGGCTCGGTACAAT-3', 5'-CGACTGCAAATACAAGTT-3'.

2.3. Cell survival detection

The viability of ASMCs under different treatment conditions was determined by the following experiments. Cell viability was assessed using Cell Counting Kit-8 (CCK-8) assay. According to the manufacturer's protocol, ASMC cells were seeded in 96-well plates at 10^5 cells/mL and 100 μL per well. After treating for a certain time, 10 μL CCK-8 solution was added to each well, and the absorbance was measured with a microplate reader (BioTek SynergyHTX, USA) at 450 nm after 3 h.

2.3.1. Effects of LPS, LY411575 and rMK on proliferation of ASMCs and determination of IC_{50} values

Cells treated with different concentrations of LPS (0.02-0.8 mg/mL) or LY411575 (0.001-0.1 mg/mL) or rMK (0-10^3 ng/mL) in 10% fetal bovine serum-containing DMEM for 24 h.

2.3.2. Effect of MK siRNA on proliferation of LPS–induced ASMCs

ASMC cells were divided into six groups according to different treatments as follows: (a) control: untreated; (b) LPS: cells treated with LPS (0.2 mg/mL) for 72 h; (c) control siRNA: cells transfected with non-target siRNA for 72 h; (d) MK siRNA: cells transfected with MK siRNA for 72 h; (e) LPS+non-target siRNA: cells transfected with non-target siRNA and stimulated with LPS (0.2 mg/mL) for 72 h; (f) LPS+MK siRNA: cells transfected with MK siRNA and stimulated with LPS (0.2 mg/mL) for 72 h.

2.3.3. Effect of LY411575 on proliferation of LPS–induced ASMCs

ASMC cells were divided into four groups according to treatment as follows: (a) control: untreated; (b) LPS: cells treated with LPS (0.2 mg/mL) for 72 h; (c) LPS+LY411575: cells stimulated with LPS (0.2 mg/mL) for 24 h and then treated with the γ-secretase inhibitor of Notch signaling LY411575 (0.03 mg/mL) and LPS for another 48 h, (d) LPS+MK siRNA: cells transfected with MK siRNA and stimulated with LPS (0.2 mg/mL) for 72 h.

2.3.4. Effect of rMK on proliferation of LPS–induced ASMCs

Cells were treated with different concentrations of LPS (0.1-0.3 mg/mL) and rMK (0.01-100 ng/mL) in 10% fetal bovine serum-containing DMEM for 48 h and 72 h.

2.4. Western blotting analysis

ASMCs were treated according to the same conditions in Section 2.3. The total protein of each group was extracted and processed according to standard procedures and quantified using the BCA method. Proteins were separated on a 12% SDS polyacrylamide gel

and transferred to a PVDF membrane. The membrane was incubated with primary antibodies [anti-MK (ab236781), 1:1 000; anti-Notch2 (ab118824), 1:1 000; and anti-β-actin (ab11003), 1:500] (Abcam, UK) at 4 ℃ overnight and then incubated with goat anti-rabbit IgG at room temperature for 1 h. The membrane was washed three times with TBST. Immunoreactive bands were visualized using an enhanced chemiluminescence detection system (Tanon 5200, China) and quantified by densitometry using ImageJ.

2.5. qPCR analysis

ASMCs were treated according to the same conditions in Section 2.3. The total RNA each group was extracted using TRizol and processed according to standard procedures. RNA concentration and purity were determined using a NanoDrop spectrophotometer. The following primers were used: *MK*, 5'-CAGACCCAGCGCATCATTG-3' (forward), 5'-TCTTGGAGGTGCAGGCTTG-3' (reverse); *Notch2*, 5'-GGTGGTCAAGAGCCCTGTGT-3' (forward), 5'-TGGCCTGCGTCACACAGTA-3' (reverse); *GAPDH*, 5'-CAGCCAGGAGAAATCAAACAG-3' (forward), 5'-GACTGAGTACCTGAACCGGC-3') (reverse). These primers were designed and synthesized by Bioengineering Co. Ltd. (Shanghai, China). Reverse transcription was performed using a reverse transcription kit (Thermo Fisher, USA). Real-time quantitative PCR was carried out using an SYBR Green PCR kit. The amplification conditions included a denaturation step at 95 ℃ for 5 min, followed by 40 cycles at 95 ℃ for 10 s and 60 ℃ for 30 s, and an extension step at 60 ℃ for 30 s. The reaction mixture contained 10 μL of the Hieff® qPCR SYBR® Green Master Mix, 0.5 μL of each primer (10 μM), 1 μL of template DNA, and 8 μL of sterile ultrapure water. Gene expression was normalized to *GAPDH* using the $2^{-\Delta\Delta Ct}$ method.

2.6. Immunofluorescence microscopy

ASMCs (10^5 cells/mL) were inoculated in a glass bottom cell culture dish (801002, NEST, China). The cells were then washed in phosphate buffered saline (PBS) three times (3 min each time), fixed in 4% paraformaldehyde for 15 min, and washed again in PBS three times as described above. Afterward, cells were permeabilized with 0.5% Triton X-100 in PBS at 4 ℃ for 15 min and washed in PBS. The slides were incubated with anti-MK and anti-Notch2 primary antibodies at 4 ℃ overnight, washed with PBST three times (3 min each time), and incubated with fluorescein-conjugated goat anti-rabbit IgG for 1 h at room temperature. Non-specific reactions were blocked with normal goat serum for 30 min at room temperature. Nuclei were counterstained with DAPI. Sections were observed under a fluorescence microscope (OLYMPUS FV1000, Japan), and the protein expression of MK and Notch2 was evaluated.

2.7. Assessment of apoptosis by flow cytometry

ASMCs (10^5 cells/mL) were seeded in six-well plates and digested with trypsin without EDTA. Cells were then resuspended in 1× binding buffer (BD Pharmingen™), incubated with 5 μL of annexin V-FITC (BD Pharmingen™) in the dark for 15 min at room temperature, then incubated with 5 μL of propidium iodide for 15 min at room temperature.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation and analyzed by one-way analysis of variance using SPSS software version 20.0. P-values of less than 0.05 are considered statistically significant.

3. Results

3.1. Effects of LPS, LY411575 and rMK on the viability of ASMCs

LPS and LY411575 significantly inhibited ASMCs' proliferation in a dose-dependent manner ($P<0.05$), with the IC_{50} values of 0.2

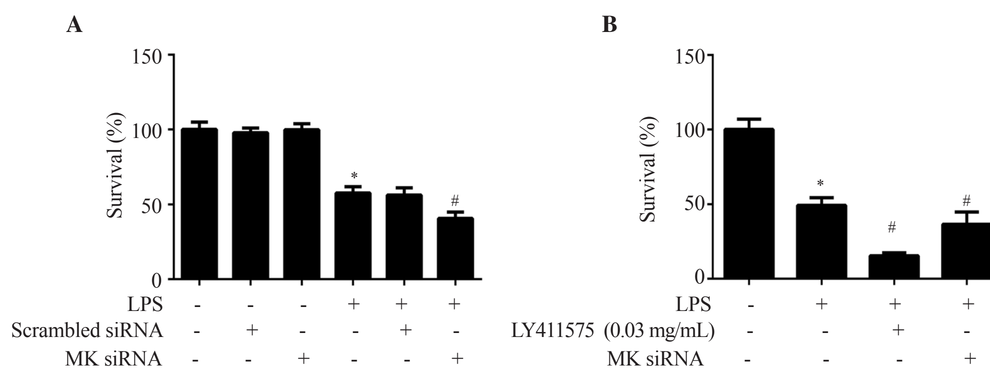

Figure 1. Effect of MK silencing (A) and LY411575 (B) on the viability of airway smooth muscle cells. Data are expressed as mean±SD of six independent experiments. *$P<0.05$ *vs.* the control. #$P<0.05$ *vs.* the LPS group. LPS: lipopolysaccharide; MK: midkine.

mg/mL and 0.03 mg/mL, respectively (Supplementary Figures 1 and 2). In contrast, rMK did not affect the survival of ASMCs (Supplementary Figure 3).

3.2. Effects of MK siRNA, LY411575 and rMK on the viability of LPS-induced ASMCs

LPS significantly decreased the viability of ASMCs, which was potentiated by MK siRNA transfection and LY411575 treatment (P<0.05) (Figure 1). In addition, rMK markedly increased the viability of LPS-treated ASMCs at 48 and 72 h (P<0.05) (Figure 2).

3.3. Effect of MK siRNA on LPS-induced MK and Notch2 protein expression

LPS significantly increased MK expression, while MK siRNA reversed this effect (P<0.05). Moreover, LPS markedly increased Notch2 expression (P<0.05), which was attenuated by MK siRNA and LY411575 (P<0.05) (Figure 3).

3.4. Effect of MK siRNA on LPS-induced MK and Notch2 mRNA expression

Similar to the results of Western blotting assay, LPS significantly increased the relative mRNA expression of *MK*, which was reduced by MK siRNA (P<0.05). Furthermore, the relative mRNA expression of *Notch2* was upregulated by LPS (P<0.05). In contrast, MK siRNA and LY411575 decreased LPS-induced *Notch2* mRNA expression (P<0.05) (Figure 3).

3.5. Immunofluorescence analysis of the MK and Notch2 expression in ASMCs

As shown in Figure 4A, LPS significantly increased MK expression

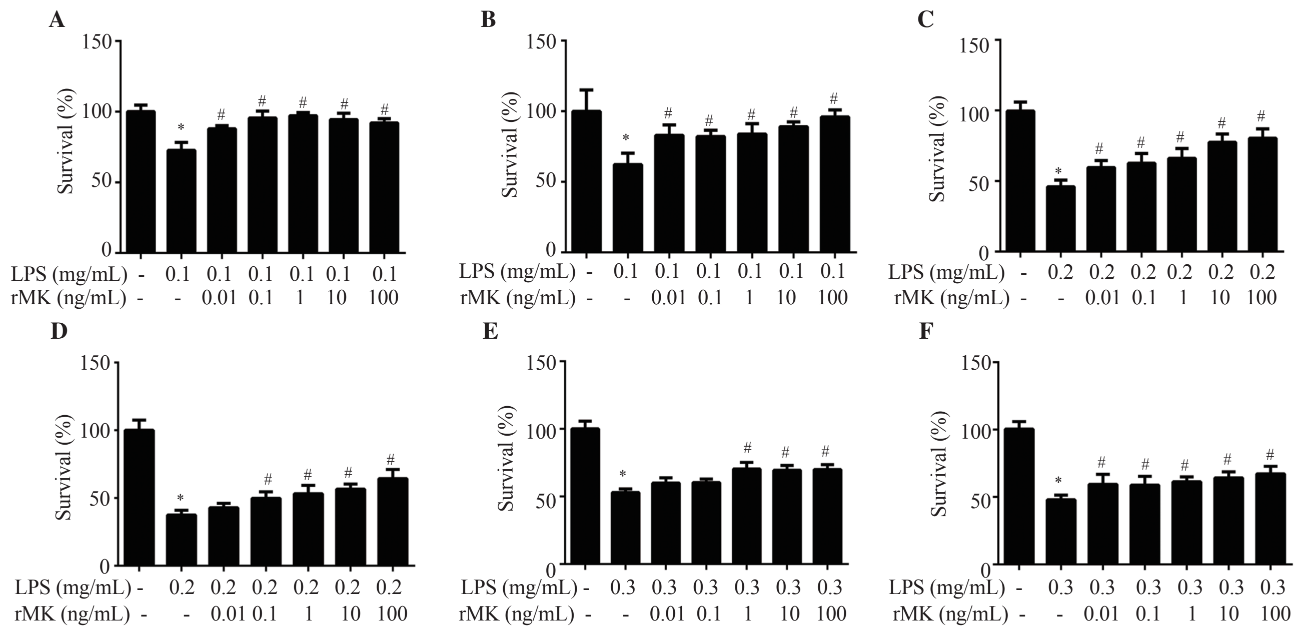

Figure 2. Effect of rMK on the viability of airway smooth muscle cells. Cells were treated with 0.1 mg/mL, 0.2 mg/mL, or 0.3 mg/mL of LPS for 48 (A, C and E) or 72 (B, D and F) h in the presence and absence of rMK. Data are expressed as mean±SD of six independent experiments. $^{*}P$<0.05 *vs.* the control. $^{\#}P$<0.05 *vs.* the LPS group. rMK: recombinant midkine.

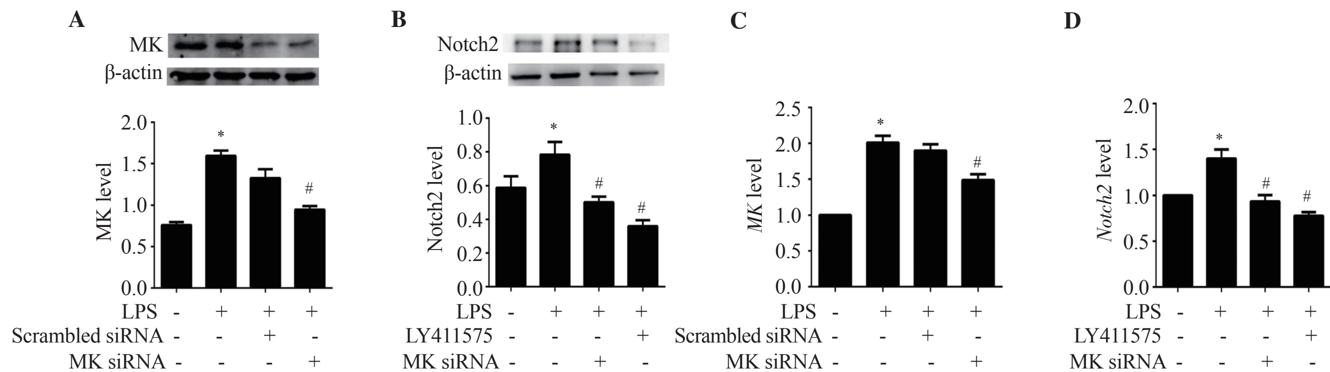

Figure 3. The protein and mRNA expression of MK (A and C) and Notch2 (B and D) in airway smooth muscle cells after MK silencing. Data are expressed as mean±standard deviation (SD) of three independent experiments. $^{*}P$<0.05 *vs.* the control. $^{\#}P$<0.05 *vs.* the LPS group.

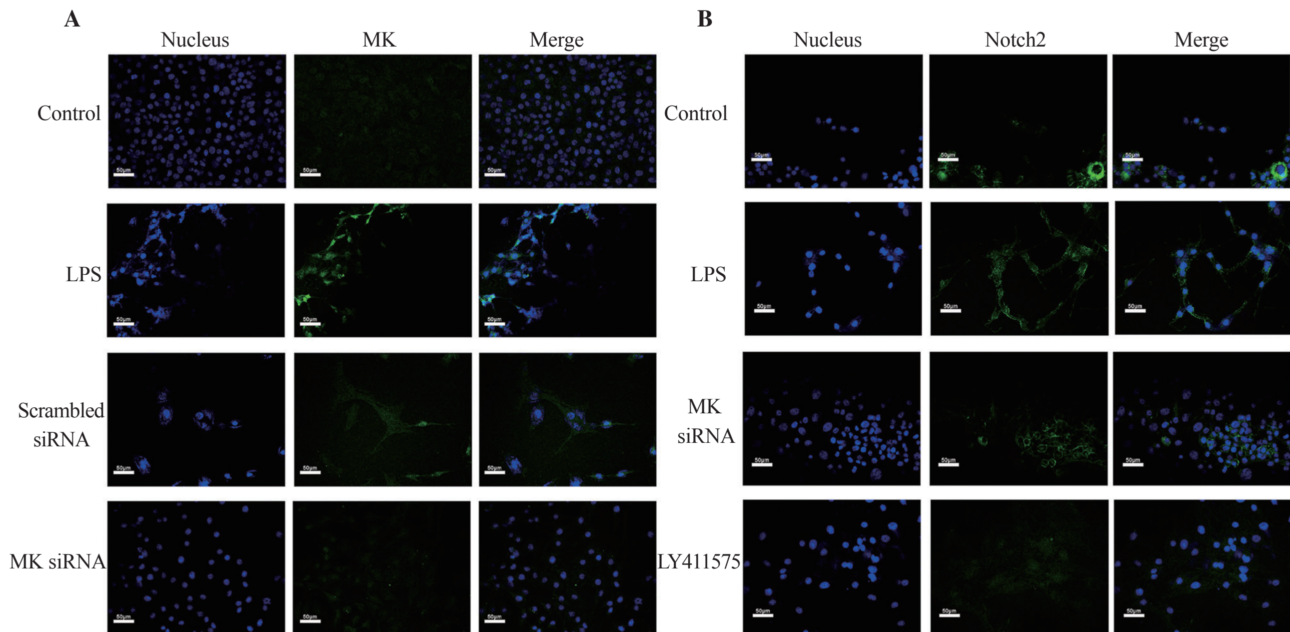

Figure 4. Immunohistochemical analysis of MK (A) and Notch2 (B) expression in airway smooth muscle cells (Magnification: × 400). Cell nuclei (blue) were stained with DAPI. MK and Notch2 (green) localized to the cytoplasm and cell membrane, respectively.

Figure 5. Effect of MK silencing and rMK on the apoptosis of airway smooth muscle cells. (A) Representative images of flow cytometric analysis. (B) Apoptotic rate. Data are expressed as mean±SD of three independent experiments. *P<0.05 *vs.* the control. #P<0.05 *vs.* the LPS group.

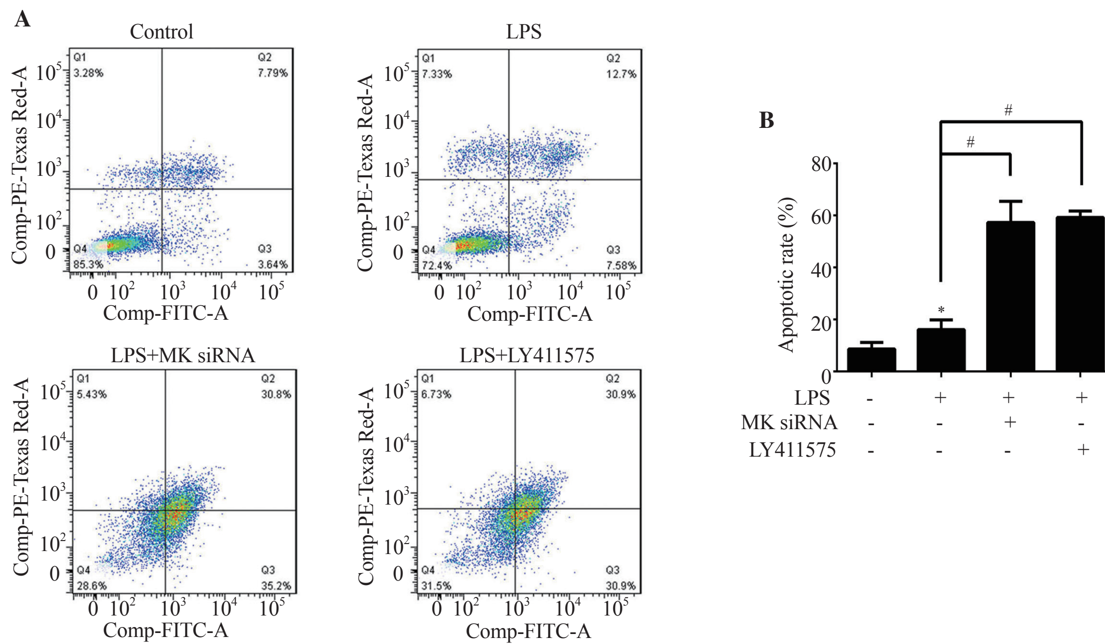

Figure 6. Effect of MK silencing and LY411575 treatment on the apoptosis of airway smooth muscle cells. (A) Representative images of flow cytometric analysis. (B) Apoptotic rate. Data are expressed as mean±SD of three independent experiments. $^{*}P<0.05$ *vs.* the control. $^{\#}P<0.05$ *vs.* the LPS group.

($P<0.01$), while MK siRNA reduced LPS-induced MK expression. The results also demonstrated that LPS prominently increased Notch2 expression, which was abrogated by MK siRNA and LY411575 ($P<0.05$) (Figure 4B).

3.6. Effects of rMK, MK siRNA, and LY411575 on the apoptosis rate of LPS–stimulated ASMCs

The apoptosis of ASMCs was determined by flow cytometry. The results exhibited that LPS significantly increased apoptosis rate, and this effect was further enhanced by MK silencing and LY411575 (Figures 5 and 6). However, rMK treatment reduced LPS-induced apoptosis (Figure 5).

4. Discussion

ALI is a clinical disease with high mortality and treatment burden. Despite great progress in diagnosis and treatment, disease prognosis is poor. ALI is characterized by the apoptosis and necrosis of vascular endothelial cells and impaired vascular integrity, leading to increased permeability and pulmonary edema. LPS is a glycolipid of the outermost membrane of Gram-negative bacteria that induces inflammatory reactions and cytokine secretion and triggers ALI in animal models[14,15]. In our study, the effect of LPS-induced ALI was assessed *in vitro*. Previous studies have found that TNF-α expression is increased in LPS-induced ALI. TNF-α reduces vascular permeability, leading to pulmonary vascular endothelial cell injury and pulmonary edema[16,17]. MK expression is low in healthy organs and increases during oxidative stress, inflammation, and tissue repair[18,19]. MK promotes the release of MCP-1, MIP-2, and other chemokines, and the aggregation of macrophages and neutrophils[20,21]. In this study, LPS increased the protein expression of MK in rat ASMCs. However, the role of MK and related signaling pathways in ASMCs in LPS-induced ALI is incompletely understood.

MK can promote cell proliferation. It is expressed in many tumors and inflammatory diseases and has anti-apoptotic effects[22–24]. The present study assessed the effect of rMK on the proliferation and apoptosis of ASMCs. In a mouse model of acid- and ventilator-induced lung injury, an increase in collagen deposition and hydroxyproline levels and a decrease in lung compliance were attenuated in $MK^{-/-}$ mice *vs.* wild-type mice[10]. The inhibition of Nox1, MK, and Notch2 attenuated epithelial-to-mesenchymal transition (EMT), demonstrating that MK plays an important role in airway remodeling[25,26]. MK enhances the hypoxia-induced proliferation and differentiation of human lung epithelial cells[27,28]. Our results showed that ASMC survival was lower in the MK siRNA group than in the LPS group, indicating that MK reduced LPS-induced ALI. In addition, LPS decreased the viability and increased apoptosis rate of ASMCs, whereas rMK attenuated these effects. These results show that MK participates in the repair of inflammatory tissues, increases ASMCs survival, and inhibits apoptosis, leading to airway remodeling, which is reversed by MK inhibition.

Notch pathway is an evolutionarily conserved pathway that regulates many cell-fate decisions during development and is regulated by receptor proteolysis. Upon proteolysis, the Notch intracellular domain travels to the nucleus and interacts with a

transcription factor complex to regulate gene expression[29,30]. The γ-secretase inhibitor of Notch signaling, LY411575, inhibits osteoclast differentiation and bone destruction through the Notch/HES1/MAPK/Akt pathway[31]. Notch2 causes tumor invasion and metastasis by regulating EMT and is downregulated by Notch inhibitors and endogenous compounds, resulting in mesenchymal-to-epithelial transition[32]. Notch2, as a receptor of MK[33,34], interacts with MK in human lung epithelial cells, and mechanical stretching can dictate MK-induced EMT. Notch2 signaling inhibition blocks EMT more effectively than endogenous MK. Silencing MK or Notch2 reduces pulmonary fibrosis[25,35].

Notch2 is required for inflammatory cytokine-driven goblet cell metaplasia in the lung, and Notch2 inhibition can be a therapeutic strategy for preventing this pathological process in airway diseases[36]. Antisense oligonucleotides downregulated Jag1 and Notch2 in goblet cell metaplasia associated with allergen-induced asthma and upregulated the ciliated cell marker FoxJ1. Moreover, the antisense oligonucleotide-mediated decrease in Jag1 and Notch 2 expression inhibited goblet cell metaplasia, mucus production, and airway remodeling[37].

Our results showed that LPS increased the mRNA and protein expression of MK and Notch2. In the MK siRNA group, these expressions were significantly decreased. MK promoted the proliferation of gastric cancer cells through the Notch signaling, whereas the cisplatin-induced apoptosis of these cells was reduced by rMK and enhanced by MK silencing[38]. Apoptosis may be induced in MK siRNA-transfected cells by an apoptosome-dependent mechanism, with downregulation of Bcl-2 expression, upregulation of Bax expression, reduction of mitochondrial membrane potential, release of cytochrome c, and activation of caspases 3, 8, and 9[38,39]. Consistent with the results of the previous study, we found that the inhibition of MK and Notch2 expression decreased the proliferation of ASMCs and promoted apoptosis, demonstrating that MK and Notch2 jointly participate in lung tissue remodeling.

There are some limitations in this study. First, siRNA can be toxic to ASMCs by inhibiting MK proliferation. Although we have avoided the toxicity of siRNA as much as possible, when LPS and siRNA act together, the cell survival rate is lower. So we will use lentivirus for transfection in future research to avoid the toxicity of siRNA. Second, this study only evaluates the role of MK in ASMCs *in vitro*. Future research should be performed *in vivo* to obtain more data to support our hypothesis and conclusions.

In conclusion, MK promotes the proliferation of ASMCs and reduces cell apoptosis in LPS-induced ALI *via* Notch2 signaling pathway. However, the functions of downstream proteins of the MK/Notch2 signaling pathway are unclear, and the role of MK can be further investigated using other gene-delivery technologies, such as lentiviral vectors.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

LX conceived the concepts. LX and SY contributed to the design and quality control of the study, HQ, DT, LL, QJ, LQ, LK, SD, XS, WH and WX performed the experiments. HQ, WB, and WY performed data analysis, statistical analysis and original manuscript preparation. LX and SY edited and reviewed manuscript. All authors read and approved the final manuscript.

References

[1] Siempos II, Choi AM. Midkine: In the middle of the pathogenesis of acute respiratory distress syndrome-associated lung fibrosis? *Am J Respir Crit Care Med* 2015; **192**(3): 271-272.

[2] Kaku S, Nguyen CD, Htet NN, Tutera D, Barr J, Paintal HS, et al. Acute respiratory distress syndrome: Etiology, pathogenesis, and summary on management. *J Intensive Care Med* 2020; **35**(8): 723-737.

[3] Krzystek-Korpacka M, Mierzchala M, Neubauer K, Durek G, Gamian A. Midkine, a multifunctional cytokine, in patients with severe sepsis and septic shock: A pilot study. *Shock* 2011; **35**(5): 471-477.

[4] Şalaru DL, Arsenescu-Georgescu C, Chatzikyrkou C, Karagiannis J, Fischer A, Mertens PR. Midkine, a heparin-binding growth factor, and its roles in atherogenesis and inflammatory kidney diseases. *Nephrol Dial Transplant* 2016; **31**(11): 1781-1787.

[5] Kishida S, Mu P, Miyakawa S, Fujiwara M, Abe T, Sakamoto K, et al. Midkine promotes neuroblastoma through notch2 signaling. *Cancer Res* 2013; **73**(4): 1318-1327.

[6] Liedert A, Mattausch L, Rontgen V, Blakytny R, Vogele D, Pahl M, et al. Midkine-deficiency increases the anabolic response of cortical bone to mechanical loading. *Bone* 2011; **48**(4): 945-951.

[7] Shimizu K, Chiba S, Saito T, Kumano K, Hamada Y, Hirai H. Functional diversitymamong notch 1, notch 2, and notch 3 receptors. *Biochem Biophys Res Commun* 2002; **291**(4): 775-779.

[8] Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y. Hes binding to STAT3 mediates crosstalk between Notch and JAK-STAT signalling. *Nat Cell Biol* 2004; **6**(6): 547-554.

[9] Nordin SL, Jovic S, Kurut A, Andersson C, Gela A, Bjartell A, et al. High

expression of midkine in the airways of patients with cystic fibrosis. *Am J Respir Cell Mol Biol* 2013; **49**(6): 935-942.

[10]Zhang R, Pan Y, Fanelli V, Wu S, Luo AA, Islam D, et al. Mechanical stress and the induction of lung fibrosis *via* the midkine signaling pathway. *Am J Respir Crit Care Med* 2015; **192**: 315-323.

[11]Dekkers BG, Schaafsma D, Nelemans SA, Zaagsma J, Meurs H. Extracellular matrix proteins differentially regulate airway smooth muscle phenotype and function. *Am J Physiol Lung Cell Mol Physiol* 2007; **292**(6): L1405-L1413.

[12]Postma DS, Timens W. Remodeling in asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2006; **3**: 434-439.

[13]Martinez FD. The origins of asthma and chronic obstructive pulmonary disease in early life. *Proc Am Thorac Soc* 2009; **6**(3): 272-277.

[14]Pan W, Meng LP, Su J, Yang ZB, Du WF, Xu ZW, et al. *Crotalaria ferruginea* extract attenuates lipopolysaccharide-induced acute lung injury in mice by inhibiting MAPK/NF-κB signaling pathways. *Asian Pac J Trop Biomed* 2021; **11**(11): 481-490.

[15]Luo W, Yang LB, Qian CC, Ma B, Manjengwa GM, Miao XM, et al. Flavokawain B alleviates LPS-induced acute lung injury *via* targeting myeloid differentiation factor 2. *Acta Pharmacol Sin* 2022; **43**(7): 1758-1768.

[16]Mukhopadhyay S, Hoidal JR, Mukherjee TK. Role of TNFα in pulmonary pathophysiology. *Respir Res* 2006; **7**(1): 125.

[17]Liu X, Jiang Y, Chen B, Qiu X, He S, Liu X, et al. Effects of membrane-type PBLs on the expression of TNF-α and IL-2 in pulmonary tissue of SD rats after LPS-induced acute lung injury. *Adv Clin Exp Med* 2022. doi: 10.17219/acem/152739.

[18]Campbell VK, Gately RP, Krishnasamy R, Burg D, Robertson GR, Gray NA, et al. Midkine and chronic kidney disease-associated multisystem organ dysfunctions. *Nephrol Dial Transplant* 2021; **36**(9): 1577-1584.

[19]Weckbach LT, Preissner KT, Deindl E, Weckbach LT. The role of midkine in arteriogenesis, involving mechanosensing, endothelial cell proliferation, and vasodilation. *Int J Mol Sci* 2018; **19**(9): 2559.

[20]Sato W, Sato Y. Midkine in nephrogenesis, hypertension and kidney diseases. *Br J Pharmacol* 2014; **171**(4): 879-887.

[21]Takada S, Sakakima H, Matsuyama T, Otsuka S, Nakanishi K, Norimatsu K, et al. Disruption of *Midkine* gene reduces traumatic brain injury through the modulation of neuroinflammation. *J Neuroinflammation* 2020; **17**(1): 40.

[22]Cai YQ, Lv Y, Mo ZC, Lei J, Zhu JL, Zhong QQ. Multiple pathophysiological roles of midkine in human disease. *Cytokine* 2020; **135**: 155242.

[23]Said EA, Al-Dughaishi S, Al-Hatmi W, Al-Reesi I, Al-Riyami M, Al-Balushi MS, et al. Human macrophages and monocyte-derived dendritic cells stimulate the proliferation of endothelial cells through midkine production. *PLoS One* 2022; **17**(4): e0267662.

[24]Ueno M, Kariya R, Gunya S, Cheevapruk K, Okada S, Ueno M. Midkine inhibitor (iMDK) induces apoptosis of primary effusion lymphoma *via* G_2/M cell cycle arrest. *Leuk Res* 2022; **116**: 106826.

[25]Huang Y, Hoque MO, Wu F, Trink B, Sidransky D, Ratovitski EA, et al. Midkine induces epithelial-mesenchymal transition through Notch2/Jak2-Stat3 signaling in human keratinocytes. *Cell Cycle* 2011; **71**(14): 5009-5019.

[26]Lu L, Zhu H, Wang H, Liang H, Hou Y, Dou H, et al. *H. sinensis* mycelium inhibits epithelial-mesenchymal transition by inactivating the midkine pathway in pulmonary fibrosis. *Front Med* 2021; **15**(2): 313-329.

[27]Weckbach LT, Groesser L, Borgolte J, Pagel JI, Pogoda F, Schymeinsky J, et al. Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. *Am J Physiol Heart Circ Physiol* 2012; **303**(4): H429- H438.

[28]Zhang H, Okamoto M, Panzhinskiy E, Zawada WM, Das M. PKCδ/midkine pathway drives hypoxia-induced proliferation and differentiation of human lung epithelial cells. *Am J Physiol Cell Physiol* 2014; **306**(7): 648-658.

[29]Artavanis-Tsakonas S, Matsuno K, Fortini ME, Artavanis-Tsakonas S. Notch signaling. *Science* 1995; **268**(5208): 225-232.

[30]Naiche LA, Villa SR, Kitajewski JK, Naiche LA. Endothelial cell fate determination: A top Notch job in vascular decision-making. *Cold Spring Harb Perspect Med* 2022; **12**(11): a041183.

[31]Chen X, Chen X, Zhou Z, Qin A, Wang Y, Fan B, et al. LY411575, a potent γ-secretase inhibitor, suppresses osteoclastogenesis *in vitro* and LPS-induced calvarial osteolysis *in vivo*. *J Cell Physiol* 2019; **234**(11): 20944-20956.

[32]Zhu F, Li T, Qiu F, Fan J, Zhou Q, Ding X, et al. Preventive effect of Notch signaling inhibition by a γ-secretase inhibitor on peritoneal dialysis fluid-induced peritoneal fibrosis in rats. *Am J Pathol* 2010; **176**(2): 650-659.

[33]Güngör C, Hofmann BT, Wolters-Eisfeld G, Bockhorn M, Güngör C. Pancreatic cancer. *Br J Pharmacol* 2014; **171**(4): 849-858.

[34]Xu JY, Chang W, Sun Q, Peng F, Yang Y, Xu JY. Pulmonary midkine inhibition ameliorates sepsis induced lung injury. *J Transl Med* 2021; **19**(1): 91.

[35]Liu P, Zhao L, Gu Y, Zhang M, Gao H, Meng Y, et al. LncRNA SNHG16 promotes pulmonary fibrosis by targeting miR-455-3p to regulate the Notch2 pathway. *Respir Res* 2021; **22**(1): 44.

[36]Danahay H, Pessotti AD, Coote J, Montgomery BE, Xia D, Wilson A, et al. Notch2 is required for inflammatory cytokine-driven goblet cell metaplasia in the lung. *Cell Rep* 2015; **10**(2): 239-252.

[37]Carrer M, Crosby JR, Sun G, Zhao C, Damle SS, Kuntz SG. Antisense oligonucleotides targeting jagged 1 reduce house dust mite-induced goblet cell metaplasia in the adult murine lung. *Am J Respir Cell Mol Biol* 2020; **63**(1): 46-56.

[38]Tian W, Shen J, Chen W, Tian W. Suppression of *midkine* gene promotes the antitumoral effect of cisplatin on human gastric cancer cell line AGS *in vitro* and *in vivo via* the modulation of Notch signaling pathway. *Oncol Rep* 2017; **38**(2): 745-754.

[39]Slutsky AS, Ranieri VM. Ventilator-induced lung injury. *N Engl J Med* 2013; **369**(22): 2126-2136.

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