Biochemical and Biophysical Research Communications 445 (2014) 48–53

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The microRNA miR-17 regulates lung FoxA1 expression during lipopolysaccharide-induced acute lung injury

Xu Zhaojun [a,1], Zhang Caiping [d,1], Cheng Lijuan [b], Hu Mei [b], Tao Huai [b], Song Lan [b,c,d,*]

[a] Cardiothoracic Surgery of the First Affiliated Hospital, Hunan University of Traditional Chinese Medicine, Changsha, Hunan 41007, China
[b] Department of Biochemistry and Molecular Biology, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China
[c] Division of Stem Cell Regulation and Application, State Key Laboratory of Chinese Medicine Powder and Medicine Innovation in Hunan (Incubation), Hunan University of Chinese Medicine, Changsha, Hunan 410208, China
[d] University of South China, College of Life Science, Department of Biochemistry and Molecular Biology, Hengyang, Hunan 421001, China

ARTICLE INFO

Article history:
Received 16 January 2014
Available online 31 January 2014

Keywords:
Acute lung injury
Alveolar type II epithelial cells
FoxA1
miR-17
Gene expression

ABSTRACT

Acute lung injury (ALI) is a severe pulmonary disease that causes a high number of fatalities worldwide. Studies have shown that FoxA1 expression is upregulated during ALI and may play an important role in ALI by promoting the apoptosis of alveolar type II epithelial cells. However, the mechanism of FoxA1 overexpression in ALI is unclear. In this study, an *in vivo* murine model of ALI and alveolar type II epithelial cells injury was induced using lipopolysaccharide (LPS). LPS upregulated FoxA1 in the lung tissue of the *in vivo* ALI model and in LPS-challenged type II epithelial cells. In contrast, miR-17 was significantly downregulated in these models. After miR-17 antagomir injection, the expression of FoxA1 was significantly increased in ALI mice. MiR-17 mimics could significantly inhibit FoxA1 mRNA and protein expression, whereas the miR-17 inhibitor could significantly increase FoxA1 mRNA and protein expression in LPS-induced type II epithelial cells. Thus, our results suggest that the downregulation of miR-17 expression could lead to FoxA1 overexpression in ALI.

1. Introduction

Acute lung injury (ALI) is a life-threatening syndrome that causes a high mortality rate worldwide. It is characterized by overwhelming lung inflammation and increased microvascular permeability. Restoration of the epithelial barrier following acute lung injury is critical for the recovery of lung homeostasis. Alveolar type II epithelial cells that secrete surfactant serve as progenitor cells for alveolar type I cells, replenish type II and type I epithelial cell populations, and spread and migrate to cover the denuded surface after lung injury [1]. Previous studies have indicated that type II epithelial cells begin to proliferate 24 h after injury and are stimulated to undergo apoptosis in response to various injuries (e.g., CS, LPS, oxidative stress, and TNF-alpha) [2–5].

Forkhead box A1 (FoxA1) is a member of the winged helix family of transcription factors and shares structural similarities with FoxA2 and FoxA3. It is well accepted that FoxA1 plays a critical role in the development and function of the lung [2,6]. Our previous studies have demonstrated that FoxA1 was induced in lung tissue and type II epithelial cells during ALI, and potentially acts as a pro-apoptotic transcription factor by inhibiting the expression of bcl2 and UCP2, indicating FoxA1 may play an important role in type II epithelial cell apoptosis in ALI [5,7,8]. However, the mechanism of FoxA1 upregulation in ALI and type II epithelial cells is unclear.

Recently, efforts to determine the genetic component of ALI/ARDS pathogenesis have investigated the involvement of miRNAs in this process. In ventilation-induced lung injury, several miRs (miR-21, miR-155, let-7, and miR-146) are upregulated and treatment of mice with antisense oligonucleotides against miR-21 before high volume tidal ventilation ameliorated the induction of pulmonary edema associated with ventilation-induced lung injury [9,10]. In contrast, miR-127 and miR-16 are downregulated in ALI [11,12]. In this study, the role of miR-17 was examined because miR-17 is essential for lung development and miR-17 suppression with anti-miR-17 treatment has been shown to significantly counteract the pathological severity of the disease in a monocrotaline-induced pulmonary hypertension rat model [13]. In addition, in our preliminary experiments, the expression of miR-17 in the lung tissue of the *in vivo* lipopolysaccharide (LPS)-induced ALI model was

* Corresponding author at: Department of Biochemistry and Molecular Biology, College of Medicine, Hunan University of Chinese Medicine, 1 Xiangzui Road, Hanpu Science and Teaching Park, Changsha, Hunan 410208, China. Fax: +86 731 85381150.
E-mail address: songlan311492@163.com (L. Song).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbrc.2014.01.108
0006-291X/

downregulated. However, the role of miR-17 on its targets has not yet been elucidated in ALI. Using Targetscan at www.targets.org and miRanda at www.microrna.org, the FoxA1 gene was found to contain putative miR-17 binding sites in its 3′UTR.

In this study, an *in vivo* murine model of ALI and type II epithelial cell injury was induced by LPS, and the expression of FoxA1 and miR-17 were investigated. The role of miR-17 in the regulation of FoxA1 gene expression was further investigated. Our results showed that FoxA1 is upregulated while miR-17 is downregulated in murine lung tissue and in type II epithelial cells after being stimulated by LPS, and that miR-17 could inhibit FoxA1 expression in type II epithelial cells. This research indicates FoxA1 overexpression could result from the inhibition of miR-17 expression in ALI.

2. Materials and methods

2.1. Ethical statement

Animal care and sacrifice were conducted according to methods approved by the Animal Care and Use Committee of Hunan University of Chinese Medicine. All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Animals and ALI model

Healthy male C57BL/6 mice aged 8–10 weeks and weighing 17.6–25.4 g were purchased from the Experimental Animal Center of Hunan University of Chinese Medicine (Changsha, China) and allowed to acclimate for 3 days before experimentation. Animals were fed rodent chow and water *ad libitum*. Mice were randomly divided into different groups: a control group with intra-tracheal instillation of 1.5 mg/kg normal saline (NS) and an ALI group with intra-tracheal instillation of 3 mg/kg LPS. Mice were anesthetized by an intraperitoneal injection of 10% chloral hydrate (YuLong Algae Co. Ltd., QingDao, China) and kept in a supine position while spontaneous breathing was monitored. Mice were sacrificed at the indicated time after injury. ALI induction was verified by pathological examination of the lung.

2.3. Lung histopathology

At necropsy (n = 3 per time-point), the left lung was excised and fixed with 4% paraformaldehyde. After 24 h the lung tissue was dehydrated with graded alcohol and embedded in paraffin at 52 °C. Sections were prepared and stained with hematoxylin and eosin for evaluation of the severity of lung injury. Each lung section was blindly assigned a lung injury score (LIS) by two pathologists using the method described by Nishina et al. [14].

2.4. miR-17 antagomir experiments

To study the potential function of miR-17 on FoxA1 expression in lung tissue of ALI mice, a miRNA antagomir strategy was adopted. Antagomirs directed against miR-17 (8 mg/kg), control antagomirs, or the solvent PBS (10 animals each) were injected intravenously (tail vein) every third day until the ALI was induced as described above. Single-stranded RNAs were synthesized by Ribo Biotech (Ribo-Bio Co., Ltd., China) with the following sequences [13]: antagomiR-17 (5′-CUACCUGCACUGUAAGCACUUUG-3′) and control antagomir (5′-AAGGCAAGCUGACCCUGAAGUU-3′). Antagomirs were solved in prewarmed phosphate-buffered saline (PBS) and injected intravenously. Tissue was snap-frozen and stored at −80 °C for RNA analysis.

2.5. Isolation of murine type II epithelial cells and induction of cell injury

Type II epithelial cells were isolated at 90–95% purity from 6-week-old mice following the procedure described by Corti and colleagues [15]. For the incubation experiments, type II epithelial cells were stimulated with LPS (1 μg/ml, *Escherichia coli* 0111:B4, Sigma). A dose of LPS was chosen that was consistent with previous reports and with our pilot study. At the indicated times after treatment, cells were harvested and mRNA and protein extracted to determine FoxA1 and miR-17 expression.

2.6. Transfection of miR-17 mimics or inhibitor

MiR-17 mimics (or anti-miR-17) and their negative control oligonucleotides (miR-NC or anti-miR-NC) were obtained from Ribo Biotech (Ribo-Bio Co., Ltd., China). The transfection were performed using Lipofectamine™ 2000 (Invitrogen, USA) according to the instructions provided by the manufacturer. The transected cells were resuspended and cultured in regular culture medium for 48–72 h before analysis.

2.7. RNA extraction and real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Five micrograms of total RNA was then used as a template to synthesize cDNA using the First Strand Synthesis Kit (Invitrogen). The cDNA from this synthesis was then used in quantitative RT-PCR analysis with the TaqMan system (ABI-Prism 7700 Sequence Detection System, Applied Biosystems) using SYBR Green dye. The following primer pairs were used: FoxA1: 5′-AGGTGTGTATTCCAGACCCG-3′ and 5′-TTG ACGGTTTGGTTTGTGTG-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5′-GGTGGTCTCCTCTGACTTCAACA-3′ and 5′-GTG GTCGTTGAGGGCAATG-3′. RT-PCR data were normalized through measuring average cycle threshold (C_t) ratios between candidate genes and the control gene, GAPDH. The formula $2^{C_t(\text{Candidate})}/2^{C_t(\text{Control})}$ was used to calculate normalized ratios.

2.8. TaqMan RT-PCR for miRNA quantification

Total RNA was isolated from the cell lines with Trizol™ (Invitrogen, USA), reverse transcribed using the Taqman™ microRNA reverse transcription kit, and subjected to real-time PCR using the TaqMan™ MicroRNA Assay kit (Applied Biosystems, USA) according to the manufacturer's instructions. Reactions were performed in triplicate using the Stratagene Mx3000 instrument. MiRNA expression was normalized to U6.

2.9. Western blot analysis

Proteins were isolated and separated by 12% SDS–PAGE and then transferred onto PVDF membranes (Schleicher & Schuell). The membranes were blocked overnight in phosphate-buffered saline (PBS) containing 10% nonfat dry milk and 0.5% Tween-20, and incubated with primary antibodies for 2 h. Horseradish peroxidase-conjugated anti-rabbit or anti-goat IgG was used as secondary antibodies. The immunoreactive bands were visualized using diaminobenzidine (DAB; Boster Biological Technology). Anti-GAPDH was used to normalize for equal amounts of proteins and to calculate the relative induction ratio. The following antibodies were used: rabbit anti-FoxA1 polyclonal antibody (Abcam), goat GAPDH monoclonal antibody (Sigma), HRP-conjugated anti-goat and anti-rabbit IgG (Boster Biological Technology).

2.10. Construction of plasmid vector

To construct a luciferase reporter vector, the FoxA13′-UTR fragment containing putative binding sites for miR-17 was amplified by PCR using the following primers: sense (5′-ACAGGCA CTGCAATACTCGCCTTACGGCTC-3′) and reverse (5′-TATCGTATTCA GAAATATTCACTTCGGATT-3′) and inserted downstream of the luciferase gene in the pLuc luciferase vector (Ambion, USA). Site-directed mutagenesis of the miR-17 target-site in the FoxA1–3′-UTR was performed using the Quick-change mutagenesis kit (Stratagene, Heidelberg, Germany). The constructs were sequenced and named pLuc-FoxA1-wt or pLuc-FoxA1-mut.

2.11. Luciferase assay

For reporter assays, type II epithelial cells were cultured in 24-well plates and each transfected with 100 ng of pLuc-FoxA1-wt or pLuc-FoxA1-mut and 50 nM of miR-17 mimics or anti-miR-17 using Lipofectamine 2000 (Invitrogen, USA). Forty-eight hours after transfection, cells were harvested and assayed with the Dual-Luciferase Reporter Assay kit (Promega, USA) according to the manufacturer's instructions.

2.12. Statistical analysis

Data in the figures and text were expressed as the mean ± SEM. Each experiment was performed at least three times, and statistical analyses were performed with a one-way ANOVA. Otherwise, representative data were shown. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Upregulation of FoxA1 and downregulation of miR-17 in lung tissue of ALI mice in LPS-induced type II epithelial cells

Histological examination by light microscopy revealed that in the control lung, alveoli were fully distended and no alveolar wall edema or congestion was found. Occasional inflammatory cells were scattered throughout the lung. In contrast, in the lung tissue from LPS-treated animals, features observed that were consistent with ALI included alveolar hemorrhage, destruction of alveolar attachment points, an increased number of interstitial cells, and extensive neutrophil infiltration (Supplementary Fig. 1A). The LIS significantly increased in the LPS group (Supplementary Fig. 1B). These results demonstrated that the induction of ALI in mice using LPS was successful.

LPS treatment led to a sustained increase in FoxA1 mRNA levels from 2 to 24 h and FoxA1 protein levels from 16 to 24 h in lung tissue of ALI mice (Fig. 1A and B). Maximal expression levels of FoxA1 mRNA and protein were observed at 24 h. However, the expression level of miR-17 was downregulated in the lung tissue of ALI mice (Fig. 1C).

Following treatment of type II epithelial cells from mice treated with LPS (1 μg/ml), FoxA1 mRNA and protein increased (Fig. 2A and B). In addition, the expression level of miR-17 was significantly downregulated in LPS-induced type II epithelial cells from mice (Fig. 2C).

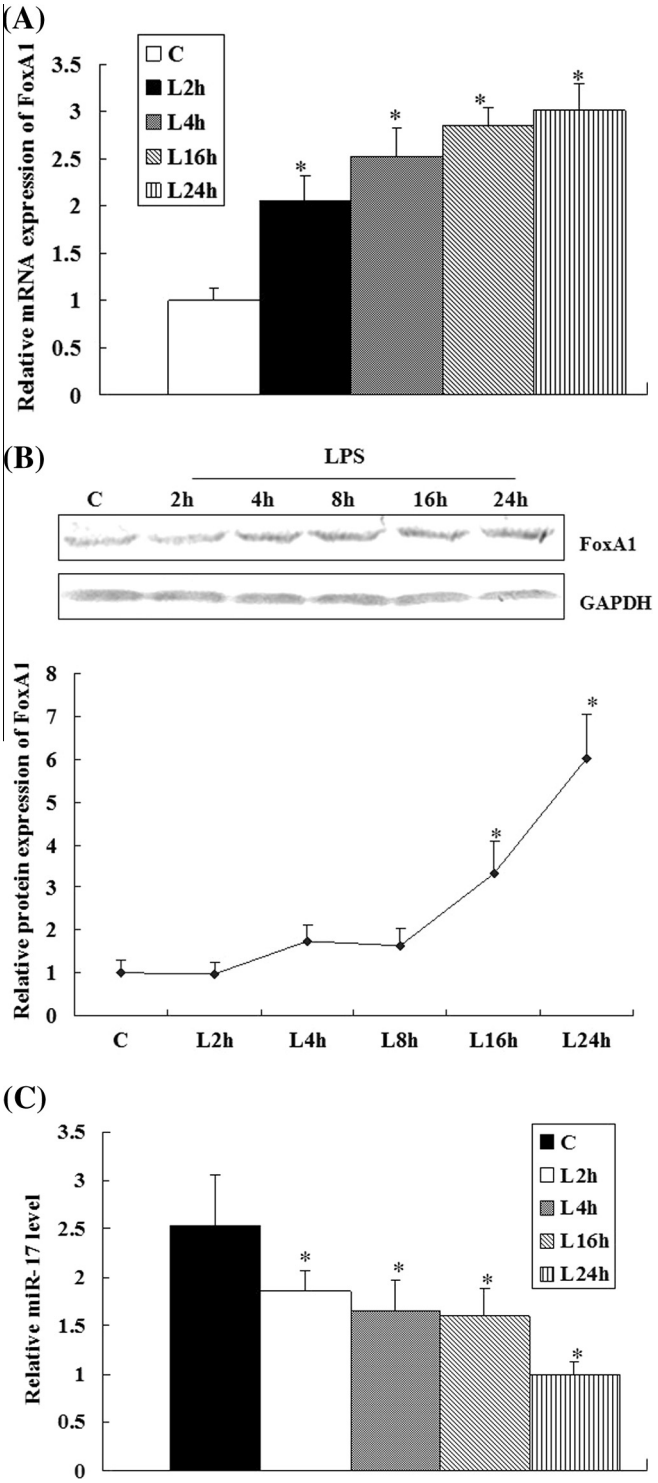

Fig. 1. Expression of FoxA1 and miR-17 in lung tissue of mice. (A) The ALI model was induced using LPS and the mRNA or protein expression of FoxA1 in lung tissues was determined by real-time PCR and Western analysis (B). (C) Levels of miR-17 were determined by real-time PCR in lung tissues of an ALI model induced by LPS. *, Statistically significant difference versus control group (C), $P < 0.05$.

3.2. FoxA1 expression upregulated in mice lung tissue by which miR-17 inhibited through antagomir

It has been reported that miRNAs can function post-transcriptionally by reducing protein yield from specific target mRNAs. Using bioinformatic analysis, FoxA1 was found to contain putative miR-17 binding sites in its 3′UTR. We speculated that the mechanism of FoxA1 overexpression might be partly due to the downregulation of miR-17 in ALI. Therefore, the role of miR-17 in the regulation of FoxA1 gene expression was further investigated.

It has been reported that miR-17 antagomirs at a concentration of 8 mg/kg significantly suppress their targeted miR in murine

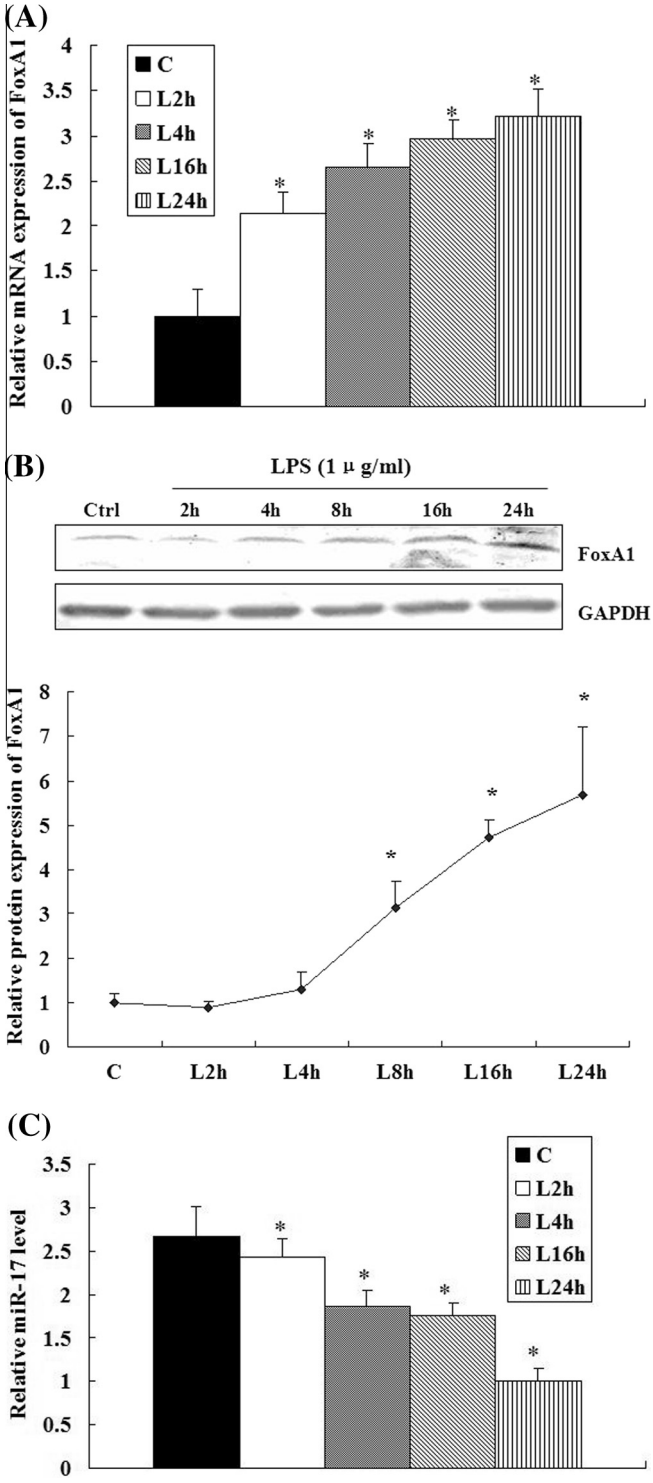

Fig. 2. Expression of FoxA1 and miR-17 in type II epithelial cells of mice. (A) Type II epithelial cells of mice were induced with LPS and the mRNA or protein expression of FoxA1 was determined by real-time PCR and Western blot (B). (C) Levels of miR-17 were determined by real-time PCR in type II epithelial cells of mice induced by LPS. C, Control group; L, LPS group. The relative values of all results were determined and expressed as mean ± SEM of three experiments in duplicate. *, Statistically significant difference versus control group (C), $P < 0.05$.

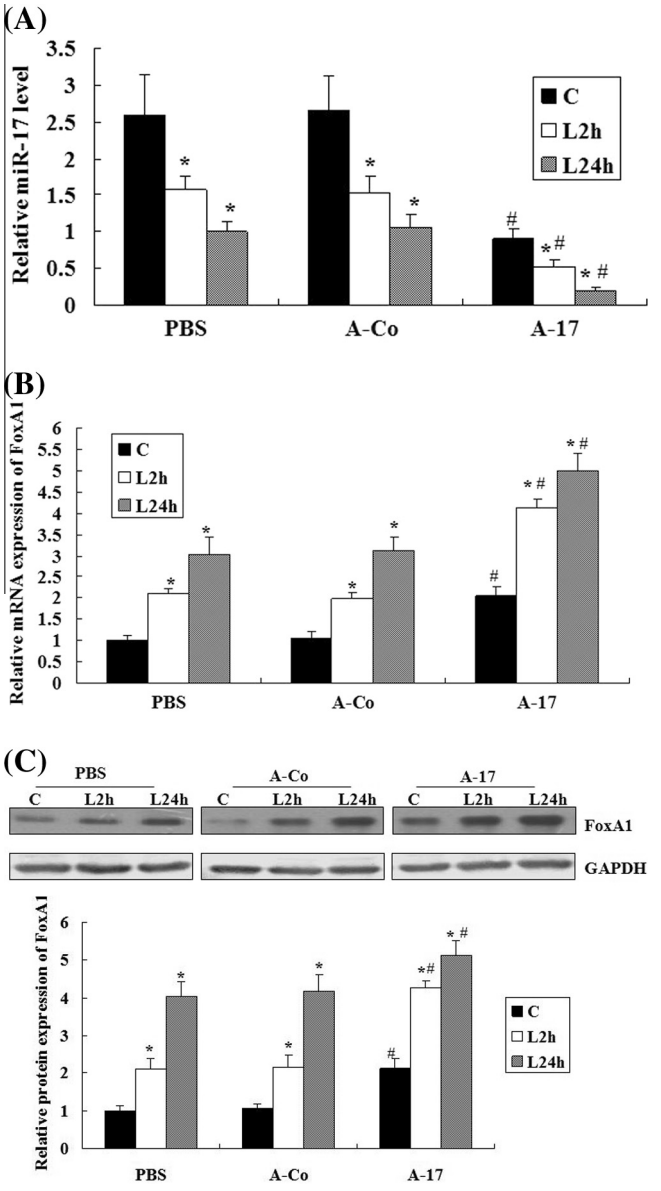

Fig. 3. FoxA1 expression is upregulated in murine lung tissue when miR-17 is inhibited through antagomirs. (A) Analysis of miR-17 expression in murine lung tissue by real-time PCR. (B) Analysis of FoxA1 mRNA and protein expression in lung tissue of mice by real-time PCR or Western analysis (C). PBS, mice treated with phosphate-buffered saline; A-Co, mice treated with antagomir control; A-17, mice treated with miR-17 antagomir. C: Control group, L: LPS group. *, Statistically significant difference versus control group (C), $P < 0.05$; #, Statistically significant difference versus PBS or A-Co group, $P < 0.05$.

lungs without affecting the expression of other miRs [13]. The effect reportedly lasts at least up to 3 days, therefore intravenous injections of antagomirs at a concentration of 8 mg/kg were started 14 days before exposure of the mice to LPS and antagomirs were injected every third day until the end of the experiment. Analysis of miR-17 expression by real-time PCR revealed an efficient knockdown in the groups treated with the miR-17 antagomir (Fig. 3A). After antagomir injection, the expression of FoxA1 significantly increased compared to PBS or antagomir-control injection for both control and LPS groups (Fig. 3B and C).

3.3. Mir-17 directly targets FoxA1 in murine type II epithelial cells

After type II epithelial cells were transfected with miR-17 mimics (or miR-NC mimics) or anti-miR-17 (or anti-miR-NC) for 48 h, real-time quantitative RT-PCR was performed to detect the expression of miR-17 in type II epithelial cells. The level of miR-17 expression in the miR-17 mimics group significantly increased by approximately 532.3% compared with the miR-NC group

(Supplementary Fig. 2). Compared with the anti-miR-NC group, the level of miR-17 expression in the anti-miR-17 group also significantly decreased by approximately 53.1% (Supplementary Fig. 2).

The correlation between miR-17 and FoxA1 expression was analyzed. After transfection of miR-17 mimics or anti-miR-17 for 48 h, type II epithelial cells were incubated with LPS. FoxA1 mRNA expression significantly decreased post-transfection with miR-17 mimics compared to NC in type II epithelial cells, while it significantly increased post-transfection with anti-miR-17 (Fig. 4A). Western blot analysis demonstrated that enforced miR-17 expression also significantly decreased or increased FoxA1 protein levels in type II epithelial cells after transfection with miR-17 mimics or anti-miR-17, respectively (Fig. 4B).

To further determine the possibility that miR-17 targets FoxA1, the miR-17 binding sequences present at the 3′UTR of FoxA1 mRNA were subcloned downstream of the firefly luciferase reporter gene in pLUC vectors and then cotransfected with either miR-17 mimics, anti-miR-17, or miR-NC into type II epithelial cells. The relative luciferase activity of the reporter containing wild-type FoxA1 3′UTR was significantly suppressed when miR-17 mimics were cotransfected, but not when it contained a mutant sequence with the miR-17 binding site (Fig. 4C). Meanwhile, there was a significant increase in luciferase activity for the reporter containing the wild-type FoxA1 3′UTR when anti-miR-17 was cotransfected, but not when it contained a mutant sequence with a miR-17 binding site. These results indicated that miR-17 suppresses FoxA1 gene expression though 3′UTR binding and silencing of FoxA1 mRNA.

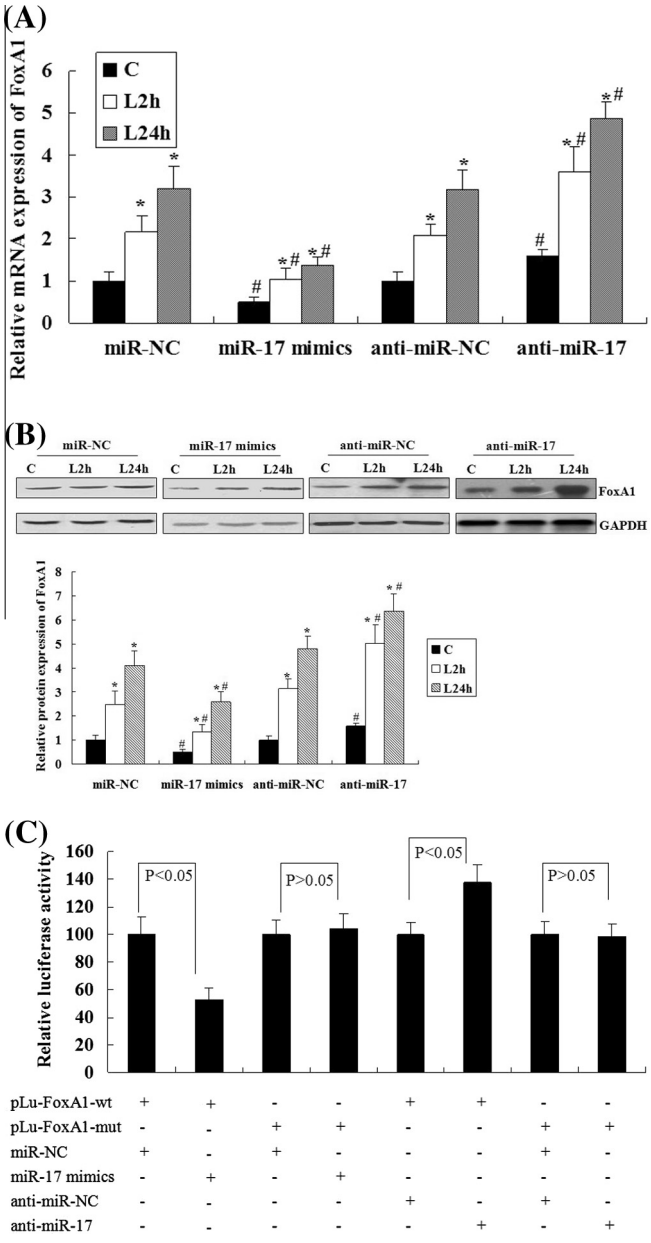

Fig. 4. Mir-17 directly targets FoxA1 in murine type II epithelial cells. 48 h after transfection with miR-17 mimics (or miR-NC mimics) or anti-miR-17 (or anti-miR-NC), type II epithelial cells were incubated with LPS, and FoxA1 mRNA or protein expression determined by Taqman real-time quantitative RT-PCR (A) and Western analysis (B). *, Statistically significant difference versus control group (C), $P < 0.05$; #, Statistically significant difference versus miR-NC(or anti-miR-NC) group, $P < 0.05$. C: Control group, L: LPS group. (C) Type II epithelial cells were co-transfected with miR-17 mimics or anti-miR-17 and pLu-FoxA1-wt or pLu-FoxA1-mut. After 48 h, the luciferase activity was measured. Values are presented as relative luciferase activity after normalization to Renilla luciferase activity.

4. Discussion

This study provides the first evidence for the upregulation of FoxA1 expression by the downregulation of miR-17 in ALI. Forkhead-box A (FoxA) family proteins are winged-helix transcription factors involved in the cell growth and differentiation of various organs such as the liver, pancreas, lung, and brain. FoxA1, a FoxA family protein, has been widely investigated in both normal development and carcinogenesis. FoxA1 has been shown to regulate signaling and transcriptional programs required for morphogenesis and cell differentiation during formation of the lung. In the lung, FoxA1 and FoxA2 are first expressed at the onset of lung bud formation and are present in the adult lung, where they are coexpressed in epithelial cells of conducting airways and in type II epithelial cells in the alveoli [16]. Studies have demonstrated that the deletion of FoxA1 causes stage-specific delays in lung maturation. Before birth, *FoxA1* deletion in the mouse lung delayed maturation of alveolar type II cells and bronchiolar Clara cells [6]. A delayed expression of both proximal and peripheral epithelial cell markers, including CCSP, SP-A, SP-B, and SP-C, in the lung was also observed in the *FoxA1*$^{-/-}$ mice [17]. Because FoxA1 regulates a number of genes critical for pulmonary homeostasis, abnormalities in the structure, function, or regulation of FoxA1 may render individuals susceptible to lung injury or disease after birth. Our previous studies have demonstrated that FoxA1 is induced in rat lung tissue during ALI and in H_2O_2-treated human type II epithelial cells. FoxA1 potentially acts as a pro-apoptotic transcription factor by inhibiting bcl-2 and UCP-2 expression, and may play an important role in the apoptosis of type II epithelial cells in ALI [5,7,8]. In this report, our results showed that the expression of FoxA1 was also upregulated in lung tissue of LPS-induced ALI mice and type II epithelial cells, consistent with our previous studies. However, the mechanisms of FoxA1 upregulation in ALI remain poorly understood.

Significant developments have been made towards understanding the contribution of microRNAs (miRNAs) in lung development and the pathogenesis of pulmonary diseases [18,19]. Studies on the role of the miR-17-92 locus during lung development have found that miRNAs at this locus are highly expressed in undifferentiated lung progenitor epithelial cells and promote their proliferation by regulating the cell cycle regulator gene Retinoblastoma-like 2 (Rbl2) [20,21]. Data have demonstrated that antagomirs of miR-17 improve heart and lung function in experimental pulmonary hypertension by interfering with lung vascular and right ventricular remodeling. miR-17 was also selected for further study because TaqMan quantitative PCR validation from our study showed a significant downregulation of miR-17 in LPS-induced or

H_2O_2-induced mice lung tissues and type II epithelial cells of mice. In addition, bioinformatic analysis showed that FoxA1 was identified as a candidate target of miR-17. Thus, the upregulation of FoxA1 likely results from the downregulation of miR-17 in ALI. Thus, the possibility of regulatory mechanisms between miR-17 and FoxA1 were further explored.

To study the potential function of miR-17 on FoxA1 expression in lung tissue of ALI mice, the expression of miR-17 was antagonized in mice using an antagomir that specifically targets miR-17. After antagomir injection the expression of FoxA1 increased compared with PBS or antagomir-control injections both in control and ALI mice. Previous studies have reported FoxA1 expression in type II epithelial cells but not in type I epithelial cells. Therefore, to investigate the effects of miR-17 on FoxA1 expression in murine type II epithelial cells, type II epithelial cells of mice were isolated and incubated with LPS (to simulate type II epithelial cell injury in ALI), and then transfected with miR-17 mimics or anti-miR-17. FoxA1 expression significantly decreased post-transfection with miR-17 mimics compared with NC in type II epithelial cells, while FoxA1 expression significantly increased post-transfection with anti-miR-17.

To study the mechanisms of miR-17 as a novel regulator of FoxA1 expression in ALI, the luciferase reporter assay was used. The results indicated that miR-17 could suppress FoxA1 gene expression though 3′UTR binding and silencing of FoxA1 mRNA. Thus, we conclude that FoxA1 is a target gene of miR-17. Taken together, we predict that miR-17 expression downregulation and FoxA1 upregulation are important in the mechanisms of acute lung injury.

MiR-17 suppresses genes that stimulate or inhibit apoptosis depending on the type of cell [22]. However, the functions of miR-17 and its targets have not been reported in ALI and type II epithelial cells. In this study, miR-17 was downregulated in ALI; furthermore, the targeting of FoxA1, a pro-apoptosis gene in ALI and type II epithelial cells, by miR-17 was validated. In conclusion, the upregulation of FoxA1 is affected by the downregulation of miR-17, which could induce type II epithelial cells apoptosis in ALI. In light of the important roles of FoxA1 and miR-17 in lung development and many lung diseases, we predict that the induction of miR-17 may be a novel therapeutic approach to ALI. In order to understand the exact functions, further investigations are needed.

Acknowledgments

This work was support by funding from the Nature Science Foundation of China (81000026), the Natural Science Foundation of Hunan Province, China (No. 14JJ7060), the Science and Technology Project of Hunan Province, China (No. 2013SK3077), the Research Foundation of Education Bureau of Hunan Province, China (No. 13C691) and the Research Foundation of Stem Cell Regulation and Application, Hunan University of Chinese Medicine, China (Nos. 2013GXB01 and 2013GXB01).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.108.

References

[1] B.D. Uhal, Cell cycle kinetics in the alveolar epithelium, Am. J. Physiol. 272 (1997) L1031–L1045.
[2] H. Kuhn, S. Kruger, S. Hammerschmidt, H. Wirtz, High concentrations of vascular endothelial growth factor reduce stretch-induced apoptosis of alveolar type II cells, Respirology 15 (2010) 343–348.
[3] L.M. Beaver, E.J. Stemmy, A.M. Schwartz, J.M. Damsker, S.L. Constant, S.M. Ceryak, S.R. Patierno, Lung inflammation, injury, and proliferative response after repetitive particulate hexavalent chromium exposure, Environ. Health Perspect. 117 (2009) 1896–1902.
[4] T.S. Lee, Y.J. Liu, G.J. Tang, H.W. Yien, Y.L. Wu, Y.R. Kou, Wood smoke extract promotes both apoptosis and proliferation in rat alveolar epithelial type II cells: the role of oxidative stress and heme oxygenase-1, Crit. Care Med. 36 (2008) 2597–2606.
[5] L. Song, B. Zhang, Y. Feng, X. Luo, X. Wei, X. Xiao, A role for forkhead box A1 in acute lung injury, Inflammation 32 (2009) 322–332.
[6] V. Besnard, S.E. Wert, K.H. Kaestner, J.A. Whitsett, Stage-specific regulation of respiratory epithelial cell differentiation by Foxa1, Am. J. Physiol. Lung Cell Mol. Physiol. 289 (2005) L750–L759.
[7] L. Song, X. Wei, B. Zhang, X. Luo, J. Liu, Y. Feng, X. Xiao, Role of Foxa1 in regulation of bcl2 expression during oxidative-stress-induced apoptosis in A549 type II pneumocytes, Cell Stress Chaperones 14 (2009) 417–425.
[8] L. Song, Z. Xu, L. Li, M. Hu, L. Cheng, L. Chen, B. Zhang, Forkhead box protein A1 inhibits the expression of uncoupling protein 2 in hydrogen peroxide-induced A549 cell line, Cell Stress Chaperon. 19 (2014) 53–60.
[9] K. Vaporidi, E. Vergadi, E. Kaniaris, M. Hatziapostolou, E. Lagoudaki, D. Georgopoulos, W.M. Zapol, K.D. Bloch, D. Iliopoulos, Pulmonary microRNA profiling in a mouse model of ventilator-induced lung injury, Am. J. Physiol. Lung Cell Mol. Physiol. 303 (2012) L199–L207.
[10] N. Yehya, A. Yerrapureddy, J. Tobias, S.S. Margulies, MicroRNA modulate alveolar epithelial response to cyclic stretch, BMC Genomics 13 (2012) 154.
[11] T. Xie, J. Liang, R. Guo, N. Liu, P.W. Noble, D. Jiang, Comprehensive microRNA analysis in bleomycin-induced pulmonary fibrosis identifies multiple sites of molecular regulation, Physiol. Genomics 43 (2011) 479–487.
[12] P. Tamarapu Parthasarathy, L. Galam, B. Huynh, A. Yunus, T. Abuelenen, A. Castillo, G. Kollongod Ramanathan, R. Cox Jr, N. Kolliputi, MicroRNA 16 modulates epithelial sodium channel in human alveolar epithelial cells, Biochem. Biophys. Res. Commun. 426 (2012) 203–208.
[13] S.S. Pullamsetti, C. Doebele, A. Fischer, R. Savai, B. Kojonazarov, B.K. Dahal, H.A. Ghofrani, N. Weissmann, F. Grimminger, A. Bonauer, W. Seeger, A.M. Zeiher, S. Dimmeler, R.T. Schermuly, Inhibition of microRNA-17 improves lung and heart function in experimental pulmonary hypertension, Am. J. Respir. Crit. Care Med. 185 (2012) 409–419.
[14] K. Nishina, K. Mikawa, Y. Takao, N. Maekawa, M. Shiga, H. Obara, ONO-5046, an elastase inhibitor, attenuates endotoxin-induced acute lung injury in rabbits, Anesth. Analg. 84 (1997) 1097–1103.
[15] M. Corti, A.R. Brody, J.H. Harrison, Isolation and primary culture of murine alveolar type II cells, Am. J. Respir. Cell Mol. Biol. 14 (1996) 309–315.
[16] V. Besnard, S.E. Wert, W.M. Hull, J.A. Whitsett, Immunohistochemical localization of Foxa1 and Foxa2 in mouse embryos and adult tissues, Gene Expr. Patterns 5 (2004) 193–208.
[17] E. Oshika, S. Liu, L.P. Ung, G. Singh, H. Shinozuka, G.K. Michalopoulos, S.L. Katyal, Glucocorticoid-induced effects on pattern formation and epithelial cell differentiation in early embryonic rat lungs, Pediatr. Res. 43 (1998) 305–314.
[18] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.
[19] M. Garofalo, G. Romano, G. Di Leva, G. Nuovo, Y.J. Jeon, A. Ngankeu, J. Sun, F. Lovat, H. Alder, G. Condorelli, J.A. Engelman, M. Ono, J.K. Rho, L. Cascione, S. Volinia, K.P. Nephew, C.M. Croce, EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers, Nat. Med. 18 (2012) 74–82.
[20] Y. Lu, J.M. Thomson, H.Y. Wong, S.M. Hammond, B.L. Hogan, Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells, Dev. Biol. 310 (2007) 442–453.
[21] Y. Lu, T. Okubo, E. Rawlins, B.L. Hogan, Epithelial progenitor cells of the embryonic lung and the role of microRNAs in their proliferation, Proc. Am. Thorac. Soc. 5 (2008) 300–304.
[22] J. Xiang, J. Wu, Feud or Friend? The Role of the miR-17-92 Cluster in Tumorigenesis, Curr. Genomics 11 (2010) 129–135.