Sox9 Activation is Essential for the Recovery of Lung Function after Acute Lung Injury

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Key Words
Acute lung injury (ALI) • Sox9 • miR-101 • Lung recovery

Abstract
Background/Aims: Acute lung injury (ALI) often predisposes acute respiratory distress syndrome (ARDS) in humans, and is featured with neutrophilic alveolitis, injury of the alveolar epithelium and endothelium, hyaline membrane formation, and microvascular thrombi. Although the pathogenesis of ALI is relatively well studied, the knowledge on the molecular regulation of the post-ALI lung recovery are poorly understood. Methods: Here, we used a widely applied bleomycin-induced ALI model to study the molecular mechanisms that underlie the post-ALI lung recovery in mice. We analyzed Sox9 expression in mouse lung by RT-qPCR, Western blot and immunohistochemistry. We analyzed miR-101 levels in mouse lung by RT-qPCR. We inhibited Sox9 in mouse lung by expressing either shRNA for Sox9 or miR-101, and analyzed the effects of Sox9 suppression on lung recovery. Results: We detected a significant increase in Sox9 protein but not mRNA, and a significant decrease in miR-101 levels in the mouse lung after ALI. MiR-101 was found to target 3'-UTR of Sox9 mRNA to inhibit its expression. Sox9 inhibition by either shRNA for Sox9 or by miR-101 further impaired the functional recovery of the lung after ALI. Conclusion: Our data suggest that Sox9 activation is essential for the recovery of lung function after ALI, which highlights a previously unappreciated mechanism that controls the post-ALI lung recovery.

Introduction

Acute lung injury (ALI) is a common pulmonary disease with clinical features of expiratory dyspnea, refractory hypoxemia and non-cardiogenic pulmonary edema, and...
could further deteriorate into highly lethal acute respiratory distress syndrome (ARDS) [1-4]. Reclassification of ALI and ARDS has been done by the Berlin definition recently [5-7]. The most important changes involve emphasis of data over the first 24 hours as well as the stratification of each oxygenation category [5-7].

The pathogenesis of ALI has been well studied, which is featured by loss of alveolar-capillary membrane integrity, excessive transepithelial neutrophil migration and release of pro-inflammatory cytokines, like interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α [8-12]. Excessive and prolonged activation of neutrophils is the key pathological feature of ALI, resulting in basement membrane destruction and permeability increases of the alveolar–capillary barrier [8-12]. Migrating neutrophils also release pro-inflammatory and pro-apoptotic cytokines to damage adjacent cells to create ulcerating lesions [8-12].

Injuries of lung endothelial cells occur during ALI, and could be partially recovered afterwards [8-12]. However, the regeneration process after ALI can be not complete, which often results in loss of some parts of respiratory capacity [8-12]. Although the pathogenesis of ALI is relatively well studied, the knowledge on the molecular regulation of the post-ALI lung recovery are poorly understood.

The transcription factor Sox9 has been extensively studied and highlighted its critical role during the developmental branching morphogenesis of embryonic lung [13-15]. Lung branching morphogenesis is a highly orchestrated process that gives rise to the complex network of gas-exchanging units in a developed lung [13-15]. Intricate regulation of signaling pathways, transcription factors, and epithelial–mesenchymal cross-talk are required to ensure the proper branching morphogenesis [13-15]. Sox9 is expressed at the distal tips of the branching epithelium in a highly dynamic manner as branching occurs and is down-regulated starting at late embryonic day, concurrent with the onset of terminal differentiation of type 1 and type 2 alveolar cells [13-15]. Fine regulation of Sox9 levels is needed for balancing proliferation and differentiation of epithelial tip progenitor cells [13-15]. Nevertheless, inactivation of Sox9 in the developing lung was also found to have minimal effects in the respiratory epithelium [16]. However, unlike in an embryonic developing lung, Sox9 could be hardly detected in the adult lung. Moreover, a role of Sox9 in the adult lung recovery has not been acknowledged previously.

MicroRNA (miRNA) is a class of non-coding small RNA of comprised of about 22 nucleotides. MiRNA has been found to regulate the gene expression post-transcriptionally, through its base-pairing with the 3′-untranslated region (3′-UTR) of target mRNA [17, 18]. It has been shown that miRNA regulates many biological events. However, a role of miRNAs, especially miR-101, in the adult lung recovery has not been studied before.

Here, we used a widely applied bleomycin-induced ALI model to study the molecular mechanisms that underlie the post-ALI lung recovery in mice. We analyzed Sox9 expression in mouse lung by RT-qPCR, Western blot and immunohistochemistry. We analyzed miR-101 levels in mouse lung by RT-qPCR. We inhibited Sox9 in mouse lung by expressing either shRNA for Sox9 or by miR-101, and analyzed the effects of Sox9 suppression on lung recovery. We detected a significant increase in Sox9 protein but not mRNA, and a significant decrease in miR-101 levels in the mouse lung after ALI. MiR-101 was found to target 3′-UTR of Sox9 mRNA to inhibit its expression. Sox9 inhibition by either shRNA for Sox9 or by miR-101 further impaired the functional recovery of the lung after ALI. Our data suggest that Sox9 activation is essential for the recovery of lung function after ALI, which highlights a previously unappreciated mechanism that controls the post-ALI lung recovery.

Materials and Methods

Animal manipulations

All mouse experiment protocols were approved by the Animal Research and Care Committee at Ruijin Hospital of Shanghai JiaoTong University School of Medicine. All experiments were performed in accordance with the guidelines from the Animal Research and Care Committee at Ruijin Hospital of Shanghai JiaoTong
University School of Medicine. Specific pathogen free (SPF) Balb/c mice (aged 12 weeks, weight 22–26 g) were purchased from the Laboratory Animal Center of Shanghai Academy of Sciences (Chinese Academy of Sciences, China). Twenty mice were used in each experimental groups.

The lung injury model was induced by intratracheal instillation of 4% bleomycin solution (Sigma-Aldrich, St Louis, MO, USA) at 20 mg/kg body weight, as has been applied and described before [8]. The control mice received intratracheal saline. No animal death was observed in the current study. Two weeks after successful induction of ALI, mice received single tail vein injection of \(10^9\) adeno-associated viruses (AAV) carrying short hairpin small interfering RNA against Sox9, or scrambled sequence as a control (scr), or recombinant miR-101 or null sequence as a control. The effects of these treatments on lung function were evaluated after another 2 weeks.

**AAV preparation and cell transfections**

Human embryonic kidney 293 cell line (HEK293) and human alveolar epithelial cell line A549 were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA), and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St Louis, MO, USA). Both cells were incubated in a humidified chamber with 5% CO\(_2\) at 37 °C.

AAV-shSox9 or AAV-miR-101 and control AAV-scr or AAV-null viruses were prepared according to general protocols. Briefly, we used a pAAV-CMV-GFP plasmid (Clontech, Mountain View, CA, USA), a packaging plasmid carrying the serotype 8 rep and cap genes, and a helper plasmid carrying the adenovirus helper functions (Applied Viromics, LLC, Fremont, CA, USA) in this study. The transgenes shSox9 or scr (as a control for shSox9) or miR-101 or null (as a control for miR-101) were inserted between CMV promoter and GFP reporter. 2A sequence was used to connect transgene and GFP to allow co-expression of both under the control of the CMV promoter. The transgenes were subcloned into the plasmid using the 50-EcoRI and 30-NheI sites of the pAAV-CMV-GFP vector. Sequencing was performed to confirm the correct orientation of the generated plasmids. AAV was prepared by triple transfection of the newly prepared plasmids, R2C8 (containing AAV2 Rep and AAV8 capsid genes) and plAd5 (containing adenovirus helper genes) into HEK293 cells by Lipofectamine 2000 reagent (Invitrogen). The viruses were purified using CsCl density centrifugation and then titered by a quantitative densitometric dot-blot assay. For cell transduction *in vitro*, the A549 cells were incubated with AAV at a MOI of 100 for 12 hours. For cell transduction *in vivo*, \(10^7\) AAV were injected from the tail vein.

**Lung function evaluation**

Lung function was measured by restrained invasive plethysmography. Mice were anesthetized, after which a small incision was made to expose the trachea, and a cannula was inserted to connect to an inline nebulizer and ventilator. Mice were then challenged with aerosolized PBS followed by increasing doses of methacholine (Sigma-Aldrich). Airway resistance index (RI) and dynamic compliance (Cdyn) were determined by analysis of pressure and flow waveforms.

**Measurement of Total Protein in bronchoalveolar Lavage fluid (BALF)**

The BALF was centrifuged at 1800 rpm for 10 minutes, the supernatant was used for measuring total protein concentration. The protein concentration was determined using the Bradford reagent (B6916, Sigma-Aldrich), with bovine serum albumin (P0834, Sigma-Aldrich) as the standard. Briefly, BAL supernatant (50 µl) was added to 1.5 ml of Bradford reagent. Absorbance at 595 nm (A-595) was recorded every minute for 3 minutes, and the mean was calculated. Protein concentration was determined from A-595 using the bovine serum albumin standard curve.

**RNA extraction, reverse transcription and quantitative RT-PCR (RT-qPCR)**

Total RNA and mi-RNA were extracted from lung or from cultured cells with miRNasy mini kit or RNeasy kit (Qiagen, Hilden, Germany), respectively. Complementary DNA (cDNA) was randomly primed from 2 µg of total RNA using the Omniscript reverse transcription kit (Qiagen). RT-qPCR was subsequently performed in triplicate with a 1:4 dilution of cDNA using the Quantitect SYBR green PCR system (Qiagen) on a Rotorgene 6000 series PCR machine. Quantitative PCR (RT-qPCR) were performed in duplicates with Quantitect SYBR Green PCR Kit (Qiagen). All primers were purchased from Qiagen. Data were collected
and analyzed with the Rotorgene software accompanying the PCR machine, using 2-ΔΔCt method for quantification of the relative mRNA expression levels. Values of genes were first normalized against α-tubulin, and then compared to controls.

Western blot

The protein from the mouse lung or cultured cells was extracted using RIPA lysis buffer (1% NP40, 0.1% Sodium dodecyl sulfate (SDS), 100μg/ml phenylmethylsulfonyl fluoride, 0.5% sodium deoxycholate, in PBS) on ice. The supernatants were collected after centrifugation at 12000×g at 4°C for 20min. Protein concentration was determined using a BCA protein assay kit (Bio-rad, China), and whole lysates were mixed with 4×SDS loading buffer (125mmol/l Tris-HCl, 4% SDS, 20% glycerol, 100μmol/l Dithiothreitol (DTT), and 0.2% bromophenol blue) at a ratio of 1:3. Samples were heated at 100 °C for 5 min and were separated on SDS-polyacrylamide gels. The separated proteins were then transferred to a PVDF membrane. The membrane blots were first probed with a primary antibody. After incubation with horseradish peroxidase-conjugated second antibody, autoradiograms were prepared using the enhanced chemiluminescent system to visualize the protein antigen. The signals were recorded using X-ray film. Primary antibodies were anti-Sox9 and anti-α-tubulin (all purchased from Cell Signaling, St Louis, MO, USA). α-tubulin was used as a protein loading control. Secondary antibody is HRP-conjugated anti-rabbit (Jackson ImmunoResearch Labs, West Grove, PA, USA). Images shown in the figure were representative from 3 repeats. Densitometry of Western blots was quantified with NIH ImageJ software. The protein levels were first normalized to loading controls, and then normalized to experimental controls.

Immunohistochemistry

Mouse lung was fixed with 4% paraformaldehyde for 4 hours, and then cryo-protected in 30% sucrose overnight. Tissue samples were sectioned in 6μM. Primary antibodies used in immunohistochemistry are rabbit polyclonal anti-Sox9 (Cell Signaling). ABC method was applied, followed by counterstaining with hematoxylin (Abcam, Cambridge, MA, USA).

Luciferase-reporter activity assay

Luciferase-reporters were successfully constructed using molecular cloning technology. Target sequence for Sox9 miRNA 3’UTR clone was purchased from Creative Biogene (Shirley, NY, USA). HEK293-miR-101, or HEK293-null, or HEK293-as-miR-101 cells were seeded in 24-well plates for 24 hours, after which they were transfected with 1μg of Luciferase-reporter plasmids per well using PEI Transfection Reagent. Luciferase activities were measured using the dual-luciferase reporter gene assay kit (Promega, Beijing, China), according to the manufacturer’s instructions.

Statistical analysis

All statistical analyses were carried out using the SPSS 18.0 statistical software package. All values are depicted as mean ± standard deviation and are considered significant if p < 0.05. All data were statistically analyzed using one-way ANOVA with a Bonferoni correction, followed by a Fisher’s exact test, as necessary.

Results

Bleomycin induces ALI in mice, resulting in a partially impaired lung function

The mouse bleomycin-ALI model has been performed as has been described before [8]. We focused on the recovery of the lung function after ALI in this model. Four weeks after bleomycin treatment, we found that by a dose-dependent increase in lung resistance index (RI, Fig. 1A) and decrease in Cdyn in response to a cholinergic stimulus (methacholine) (Fig. 1B), and a significant increase in BALF protein (Fig. 1C). These data suggest that bleomycin-ALI results in a partially impaired lung function.

Sox9 protein but not mRNA, significantly increases in bleomycin-treated mouse lung

We then analyzed Sox9 levels in the mouse lung 4 weeks after bleomycin treatment. We did not detect changes in Sox9 mRNA in the mouse lung 4 weeks after bleomycin treatment.
Fig. 1. Bleomycin induces ALI in mice, resulting in a partially impaired lung function. (A-B) Four weeks after bleomycin treatment, we found that by a dose-dependent increase in lung resistance index (RI, A) and decrease in Cdyn in response to a cholinergic stimulus (methacholine) (B). (C) Total protein in BALF. *p<0.05. N=20. Statistics: one-way ANOVA, followed by a Fish's exact test.

Fig. 2. Sox9 protein but not mRNA, significantly increases in bleomycin-treated mouse lung. (A-C) Sox9 levels in the mouse lung 4 weeks after bleomycin treatment were analyzed by RT-qPCR (A), by Western blot (B), and by immunohistochemistry (C). NS: non-significant. N=20. Statistics: one-way ANOVA, followed by a Fish's exact test. Scale bar is 50µm.

(Fig. 2A), but a significant increase in Sox9 protein by Western blot (Fig. 2B). Moreover, the increases in Sox9 protein were confirmed by immunohistochemistry (Fig. 2C). These data not only suggest that Sox9 protein but not mRNA, significantly increases in bleomycin-treated mouse lung, but also suggest that the alteration of Sox9 levels may result from translational control, rather than from gene activation at transcription level.

**Sox9 activation is essential for recovery of lung function after ALI**

In order to evaluate the role of Sox9 activation in the recovery of lung function after ALI, we inhibited Sox9 in mouse lung by injecting AAV expressing either shRNA for Sox9 or scr as a control two weeks after bleomycin treatment, and analyzed the effects of Sox9 suppression on lung recovery after another 2 weeks.
First, we confirmed the effects of Sox9 knockdown by shSox9 in A549 cells. The virus also carries a GFP reporter to allow visualization or purification of the transduced cells in vitro (Fig. 3A). (B) A549 cells that were transduced with AAV-shSox9 significantly decreased Sox9 levels. (C-F) We inhibited Sox9 in mouse lung by injecting AAV expressing either shRNA for Sox9 or scr as a control two weeks after bleomycin treatment, and analyzed the effects of Sox9 suppression on lung recovery after another 2 weeks. (C-D) Application of these viruses to bleomycin-treated mouse significantly decreased Sox9 levels in the lung, by RT-qPCR (C), and by Western blot (D). (E-F) Sox9 knockdown by shSox9 resulted in a significant increase in RI (E) and a significant decrease in Cdyn in response to a high dose methacholine (F). (G) Total protein in BALF. *p<0.05. N=20. Statistics: one-way ANOVA, followed by a Fish’s exact test.

First, we confirmed the effects of Sox9 knockdown by shSox9 in A549 cells. The virus also carries a GFP reporter to allow visualization or purification of the transduced cells in vitro (Fig. 3A). We found that A549 cells that were transduced with AAV-shSox9 significantly
decreased Sox9 levels (Fig. 3B). Application of these viruses to bleomycin-treated mouse significantly decreased Sox9 levels in the lung, by RT-qPCR (Fig. 3C), and by Western blot (Fig. 3D). Moreover, Sox9 knockdown by shSox9 resulted in a significant increase in RI (Fig. 3E), a significant decrease in Cdyn in response to a high dose methacholine (Fig. 3F) and a significant increase in BALF protein (Fig. 3G). These data suggest that Sox9 activation is essential for recovery of lung function after ALI.

**MiR-101 targets 3’-UTR of Sox9 mRNA to inhibit its expression in mouse lung**

Since we found that Sox9 protein but not mRNA, significantly increased in bleomycin-treated mouse lung, suggesting that the alteration of Sox9 levels may result from translational control, rather than from gene activation at transcription level. Thus, we hypothesize that Sox9 expression in the ALI-lung may be regulated by miRNAs. Thus, we performed bioinformatics analysis of Sox9 target sequence. Our data suggest that the miR-101 binding sites in the Sox9 mRNA sequence 3’UTR ranged from 453th base site to 459th base site (Fig. 4A). Interestingly, we found that miR-101 levels in the mouse lung significantly decreased 2 weeks after bleomycin treatment (B). (C-E) A549-miR-101, A549-null (as a control) and A549-antisense (as)-miR-101 cells were then similarly prepared by AAV transduction (C) and confirmed of miR-101 modification (D). (E) These cells were then transfected with 1μg of Sox9-3’UTR luciferase-reporter plasmid. The luciferase activities in these cells suggest that miR-101 targets 3’UTR of Sox9 mRNA to inhibit its expression. *p<0.05. N=20. Statistics: one-way ANOVA, followed by a Fish’s exact test.
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Sox9 suppression by miR-101 impairs recovery of lung function after ALI

In order to further evaluate the role of miR-101 in Sox9-dependent recovery of lung function after ALI, we inhibited Sox9 in mouse lung by injecting AAV expressing either miR-101 or null as a control two weeks after bleomycin treatment, and analyzed the effects of Sox9 suppression on lung recovery after another 2 weeks. (A) The levels of miR-101 in the mouse lung. (B) The levels of Sox9 proteins by Western blot. (C) RI. (D) Cdyn. (E) Total protein in BALF. *p<0.05. N=20. Statistics: one-way ANOVA, followed by a Fish’s exact test.

Sox9 suppression by miR-101 impairs recovery of lung function after ALI

In order to further evaluate the role of miR-101 in Sox9-dependent recovery of lung function after ALI, we inhibited Sox9 in mouse lung by injecting AAV expressing either miR-101 or null as a control two weeks after bleomycin treatment, and analyzed the effects of Sox9 suppression on lung recovery after another 2 weeks.

First, we confirmed the effects of Sox9 knockdown by miR-101 in the mouse lung. We found that application of these viruses to bleomycin-treated mouse significantly increased miR-101 levels (Fig. 5A), and significantly decreased Sox9 proteins levels by Western blot (Fig. 5B), resulting in a significant increase in RI (Fig. 5C), a significant decrease in Cdyn in response to a high dose methacholine (Fig. 5D) and a significant increase in BALF protein (Fig. 5E). Together, these data suggest that Sox9 activation is essential for recovery of lung function after ALI, possibly through miR-101 suppression (Fig. 6).
Discussion

Characterized by refractory hypoxemia and respiratory distress, ALI and ARDS are common postoperative complications, which have high incidence and mortality to be one of the leading causes for patient death [8-12]. The current ALI therapy mainly applies supportive treatments, such as protective ventilation. Although the supportive treatments may be effective to less severe subjects, their effects on severe cases are fairly poor [1-4]. Hence, better comprehension of the molecular pathogenesis of ALI is highly needed [8-12]. However, the present studies have been largely focusing on the inflammatory pathogenesis of ALI and ARDS, the efforts on the improvement of lung recovery are highly needed.

Here, we used a well-established bleomycin-ALI model in mice to address to this question. We put our focus on Sox9, since Sox9 has a well-known role in the lung development but hardly detected in the adult lung [13-15]. Embryonic lung development is instructed by crosstalk between mesenchyme and epithelia, which results in activation of transcriptional factors, such as Sox9, in a temporospatial manner [13-15]. Sox9 is expressed in both distal lung epithelium and proximal lung mesenchyme [13-15]. Interestingly, transgenic mice lacking Sox9 expression were unable to breathe and died at birth, with noticeable tracheal defects [13-15]. The tracheal phenotype was increasingly severe, with longer duration of deletion. Lymphatic vasculature was underdeveloped in the mutant trachea: Prox1, Lyve1, and Vegfr3 were decreased after Sox9 knockout. Hence, the appropriate temporospatial expression of Sox9 in lung mesenchyme is necessary for appropriate tracheal cartilage formation, lymphatic vasculature system development, and epithelial differentiation [13-15]. Since the recovery of an injured tissues always activate embryogenesis-like approaches to enhance tissue regeneration and remodeling, we are prompted to examine the changes in Sox9 in ALI models.

We first analyzed Sox9 levels in the mouse lung after bleomycin treatment. Although we did not detect changes in Sox9 mRNA, we did detect a significant increase in Sox9 protein. Thus, the gene transcription of Sox9 may be not adapted by ALI, while the translational control could occur. Since mRNAs are often regulated by miRNAs, we did bioinformatics analysis of Sox9 target sequence. Among several miRNAs that target 3'UTR of Sox9 mRNA sequence [17, 18], we only detected significant changes of miR-101 in the injured lung. This finding is consistent with a previous study, showing that silencing of microRNA-101 prevents IL-1β-induced extracellular matrix degradation in chondrocytes, in which miR-101 has been found to bind to the 3'UTR of Sox9 in rat chondrocytes [19]. Moreover, functional analysis in that study showed that miR-101 could aggravate chondrocyte ECM degradation, whereas miR-101 inhibition could reverse IL-1β-induced ECM degradation [19].

In order to evaluate the role of Sox9 activation and miR-101 as a regulator of Sox9 in the recovery of lung function after ALI, we inhibited Sox9 in mouse lung by either siRNA for Sox9 or miR-101. Both approaches resulted in further impairments of lung recovery after ALI. Hence, we conclude that Sox9 activation is essential for recovery of lung function after ALI, possibly through miR-101 suppression.

These data are pretty solid to highlight a critical role of miR-101-regulated Sox9 in the lung recovery. In line with these results, approaches that may enhance Sox9 levels or suppressing miR-101 levels possibly through use of antisense of miR-101, may improve lung function recovery. In future, these experiments may be performed as gain-of-function
experiments to substantialize the loss-of-function experiments in the current study. These approaches may greatly improve our understandings of the molecular mechanisms underlying the pulmonary functional recovery after ALI, and may shed light on development of novel therapies.

Disclosure Statement
The authors have declared that no conflict of interest exists.

References
1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE: Acute respiratory distress in adults. Lancet 1967;2:319-323.
2. Herold S, Gabrielli NM, Vadasz I: Novel concepts of acute lung injury and alveolar-capillary barrier dysfunction. Am J Physiol Lung Cell Mol Physiol 2013;305:L65-681.
3. Dengler V, Downey GP, Tuder RM, Eltzschig HK, Schmidt EP: Neutrophil intercellular communication in acute lung injury. Emerging roles of microparticles and gap junctions. Am J Respir Cell Mol Biol 2013;49:1-5.
4. Vlaar AP, Juffermans NP: Transfusion-related acute lung injury: A clinical review. Lancet 2013;382:984-994.
5. Kangelaris KN, Calfee CS, May AK, Zhuo H, Matthay MA, Ware LB: Is there still a role for the lung injury score in the era of the berlin definition ards? Ann Intensive Care 2014;4:4.
6. Khemani RG, Wilson DF, Esteban A, Ferguson ND: Evaluating the berlin definition in pediatric ards. Intensive Care Med 2013;39:2213-2216.
7. Thompson BT, Matthay MA: The berlin definition of ards versus pathological evidence of diffuse alveolar damage. Am J Respir Crit Care Med 2013;187:675-677.
8. Ji Y, Gao F, Sun B, Hao J, Liu Z: Angiotensin-converting enzyme 2 inhibits apoptosis of pulmonary endothelial cells during acute lung injury through suppressing smad2 phosphorylation. Cell Physiol Biochem 2015;35:2203-2212.
9. Yin X, Liang Z, Yun Y, Pei L: Intravenous transplantation of bmp2-transduced endothelial progenitor cells attenuates lipopolysaccharide-induced acute lung injury in rats. Cell Physiol Biochem 2015;35:2149-2158.
10. Uhlig S, Yang Y, Waade J, Wittenberg C, Babendreyer A, Kuebler WM: Differential regulation of lung endothelial permeability in vitro and in situ. Cell Physiol Biochem 2014;34:1-19.
11. Landgraf MA, Silva RC, Correa-Costa M, Hyane MI, Carvalho MH, Landgraf RG, Camara NO: Leptin downregulates lps-induced lung injury: Role of corticosterone and insulin. Cell Physiol Biochem 2014;33:835-846.
12. Cai DS, Zhou H, Liu WW, Pei L: Protective effects of bone marrow-derived endothelial progenitor cells and houttuynia cordata in lipopolysaccharide-induced acute lung injury in rats. Cell Physiol Biochem 2013;32:1577-1586.
13. Turcatel G, Rubin N, Menke DB, Martin G, Shi W, Warburton D: Lung mesenchymal expression of sox9 plays a critical role in tracheal development. BMC biology 2013;11:117.
14. Rockich BE, Hrycaj SM, Shih HP, Nagy MS, Ferguson MA, Kopp JL, Sander M, Wellik DM, Spence JR: Sox9 plays multiple roles in the lung epithelium during branching morphogenesis. Proc Natl Acad Sci U S A 2013;110:E4456-4464.
15. Moniot B, Biau S, Faure S, Nielsen CM, Berta P, Roberts DJ, de Santa Barbara P: Sox9 specifies the pyloric sphincter epithelium through mesenchymal-epithelial signals. Development 2004;131:3795-3804.
16. Perl AK, Kist R, Shan Z, Scherer G, Whitsett JA: Normal lung development and function after sox9 inactivation in the respiratory epithelium. Genesis 2005;41:23-32.
17. Di Leva G, Croce CM: Mirna profiling of cancer. Curr Opin Genet Dev 2013;23:3-11.
18. Pereira DM, Rodrigues PM, Borralho PM, Rodrigues CM: Delivering the promise of mirna cancer therapeutics. Drug Discov Today 2013;18:282-289.
19. Dai L, Zhang X, Hu X, Zhou C, Ao Y: Silencing of microrna-101 prevents il-1beta-induced extracellular matrix degradation in chondrocytes. Arthritis Res Ther 2012;14:R268.