microRNA-145-5p attenuates acute lung injury via targeting ETS2

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Research

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Abstract

Objective

Previously, the protective effect of microRNA (miR)-145-5p has been discovered in acute lung injury (ALI). Thus, this study attempts to further discuss the mechanism of miR-145-5p in ALI through the downstream E26 transformation-specific proto-oncogene 2 (ETS2)/transforming growth factor β 1 (TGF- β 1)/Smad pathway.

Methods

A lipopolysaccharide (LPS)-induced rat ALI model was established. Recombinant adenovirus miR-145-5p and/or ETS2 overexpression plasmid was administrated into rats. Afterwards, pathological damage in the lung tissue, wet/dry (W/D) ratio, apoptosis and contents of serum inflammatory factors were observed. miR-145-5p, ETS2, TGF- β 1, Smad2/3, phosphorylated Smad2/3 levels were measured in rats.

Results

miR-145-5p was down-regulated, ETS2 was up-regulated and TGF- β 1/Smad pathway was activated in LPS-suffered rats. Overexpression of miR-145-5p inactivated the TGF- β 1/Smad pathway and attenuated ALI, as reflected by relieved pathological damage, and decreased W/D ratio, apoptosis and inflammatory response. Oppositely, loss of miR-145-5p or enhancement of ETS2 worsened ALI and activated the TGF- β 1/Smad pathway. Moreover, elevation of ETS2 decreased miR-145-5p-mediated protection against ALI.

Conclusion

Evidently, miR-145-5p negatively regulates ETS2 expression and inactivates TGF- β 1/Smad pathway to ameliorate ALI in rats.

Introduction

Acute lung injury (ALI), a moderate form of acute respiratory distress syndrome (ARDS), is a continuum of lung changes caused by multiple lung injuries [1]. ALI/ARDS, characterized by pulmonary edema, diffuse alveolar injury, surfactant dysfunction and inflammation, clinically complains reduced lung compliance, severe hypoxemia and bilateral lung infiltration [2]. Sepsis, trauma or multiple traumas are the defined risk factors that increase alveolar epithelial endothelial permeability, flood alveoli, and reduce lung compliance [3, 4]. Ventilator management has the most important impact on mortality, but fluid management and timely source control are fundamental in the treatment and prevention of ALI[5]. The long-term sequelae of ARDS include long-term cognitive impairment, mental illness, neuromuscular

weakness and pulmonary insufficiency [6]. Therefore, it is still a considerable challenge to improve the strategic efficacy of ALI/ARDS.

MicroRNAs (miRNAs), small noncoding RNAs that are highly evolutionary conserved, have been closely implicated in the pathogenesis of ALI, and miRNA-based therapy has a promising potential in the treatment of ALI [7]. Some types of miRNA have suggested protective effects on *in vivo* lipopolysaccharide (LPS)-induced ALI models, such as miR-802 [8], miR-377-3p [9] and miR-27a [10]. Also, miR-145 displays a decrease in ALI [11], and up-regulation of miR-145 presents a satisfactory efficacy to suppress inflammation in ALI [12]. As to miR-145-5p, it has been recently discussed to be protective for ALI through limiting oxidative stress and inflammation [13]. E26 transformation-specific proto-oncogene 2 (ETS2) was analyzed as a target of miR-145-5p, which has been formerly investigated to regulate inflammation in ALI [14]. ETS2 is an ubiquitous transcription factor that is involved in pulmonary fibrosis (PF) [15]. As informed, miR-145 could negatively regulate transforming growth factor β (TGF- β)/Smad signaling pathway to ameliorate PF [16]. TGF- β 1 directly activates Smad signaling and deregulated TGF- β /Smad signaling pathway is a pathogenic mechanism in tissue fibrosis [17]. Actually, the inactivation of TGF- β 1/Smad signaling pathway could attenuate ALI [18] and PF [19]. However, the mechanism by which miR-145-5p targets ETS2 and regulates TGF- β 1/Smad signaling pathway in ALI is obscure. Thus, this research attempted to further discuss the mechanism of miR-145-5p in ALI through the downstream ETS2/TGF- β 1/Smad pathway.

Methods And Materials

Ethics statement

This study was approved by the Animal Care and Use Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Adenovirus delivery

Fifty healthy male C57BL/6 rats (8–10 weeks old) of specific pathogen-free (SPF) grade were purchased from Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, and they were raised in separate ventilated cages at 22–25°C and randomly divided into 10 groups (5 rats/group). Recombinant adenoviruses, Ad-miR-145-5p and Adas-miR-145-5p (Obio, Shanghai, China), as well as adenoviruses that did not express the transgene (Ad-GFP and Adas-GFP) were intratracheally injected into rats 7 d before ALI induction at 1×10^9 pfu [20].

Animal treatment

Rats were grouped as follows: control group (intraperitoneal injection of normal saline); LPS group (intraperitoneal injection of LPS), LPS + Ad-GFP group (intraperitoneal injection of LPS after intratracheal injection of Ad-GFP), LPS + Ad-miR-145-5p (intraperitoneal injection of LPS after intratracheal injection of Ad-miR-145-5p); LPS + Adas-GFP group (intraperitoneal injection of LPS after intratracheal injection of Adas-GFP); LPS + Adas-miR-145-5p (intraperitoneal injection of LPS after intratracheal injection of Adas-

miR-145-5p); LPS + oe-NC group (intraperitoneal injections of LPS and 50 mg/L oe-NC plasmid); LPS + oe-ETS2 group (intraperitoneal injections of LPS and 50 mg/L oe-ETS2 plasmid); LPS + Ad-miR-145-5p + oe-NC group (intraperitoneal injection of LPS after intratracheal injection of Adas-miR-145-5p, and further intraperitoneal injection of 50 mg/L oe-NC plasmid); LPS + Ad-miR-145-5p + oe-ETS2 group (intraperitoneal injection of LPS after intratracheal injection of Adas-miR-145-5p, and further intraperitoneal injection of 50 mg/L oe-ETS2 plasmid). The dose of normal saline and LPS (Sigma Aldrich, MI, USA) was 30 mg/kg. Oe-NC and oe-ETS2 were provided by Genechem (Shanghai, China). All rats were fed normally with water supply and alternate light/dark cycles of 12 h [21].

Weight/dry (W/D) ratio

After euthanasia, the right lungs of rats were excised and baked at 80°C for 48 h to obtain the dry weight. The ratio of W/D was calculated [22].

Hematoxylin-eosin (H&E) staining and Masson staining

The lung sections were fixed with 10% formalin, stored in paraffin overnight, and cut into 4 µm. H&E staining: the sections were stained with H&E solutions, treated with gradient ethanol, permeabilized with xylene and then mounted. Masson staining: the sections were stained with a red algae fuchsin solution, washed with glacial acetic acid, and immersed in phosphomolybdic acid. Next, the sections were dyed with aniline blue solution, soaked in glacial acetic acid, treated with xylene and observed under the electron microscope [23].

Enzyme-linked immunosorbent assay (ELISA)

The levels of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) in the serum of rats were detected using a quantitative factor ELISA kit (ab100704 and ab201279; Abcam, MA, USA) [24].

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) staining

The proportion of apoptotic cells in the lung paraffin-embedded sections was determined using a detection kit (Roche Molecular, CA, USA). Cells with brown nuclei were apoptotic cells. The proportion of apoptotic cells was analyzed using Image-Pro Plus 5.0 (Media Cybernetics, MA, USA). Each section was observed in 10 random fields by double blind method [25].

Western blot assay

Lysed with radioimmunoprecipitation lysis buffer and phenyl methyl sulfonyl fluoride (Beyotime, Shanghai, China), the protein sample was quantified by a bicinchoninic acid protein detection kit (Boster, Hubei, China). The protein sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, printed on the membrane (Millipore, USA) and blocked. The primary antibodies ETS2 (1:200, P15036-ETS2_HUMAN, Santa Cruz Biotechnology, CA, USA), TGF-β1 (1:1000, P01137-TGFB1_HUMAN, R&D Systems, MN, USA), Smad2/3 (1:1000, Q15796-SMAD2_HUMAN, CST, MA, USA) and β-actin (1:1000, P60711-ACTB_RAT, Santa Cruz Biotechnology) were incubated with the blots overnight,

so did enzyme-labeled secondary antibody (CST) for 2 h. Finally, the blots were developed with enhanced chemiluminescence agents and analyzed by Image J (NIH, MD, USA) [26].

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The lung tissues of rats were treated with the kit (16096020; Thermo Fisher Scientific, NY, USA) to extract total RNA. The primers (Table 1) were designed using biological software Premier 5 and oligo 6, and synthesized by Takara (Kyoto, Japan). U6 was an internal control of miR-145-5p, and β -actin was that of ETS2, TGF- β 1 and Smad2/3. The concentration and purity of RNA were determined by an ultraviolet spectrophotometer. Complementary DNA was produced, and performed RT-qPCR with SYBR Green (RR091A; Takara) in ABI 7500 Real-Time PCR system (Applied Biosystems, CA, USA). The $2^{-\Delta\Delta C_t}$ method was used to calculate gene mRNA level.

Table 1
Primer sequences

Genes	Primer sequences (5'-3')
miR-145-5p	F: TCGGCAGGGTCCAGTTTTCCCA
	R: CTCAACTGGTGTCGTGGA
TGF- β 1	F: ACCTGTGCAAATCCATG
	R: GCCAACCATGCTCAACTTCG
Smad2/3	F: TGCAGTTTTGTTCCAGGAATACAT
	R: CTTTGAAGATGTCAGAGTCAAGCAG
ETS2	F: AAGAACCCCTGGCTGGCTGTGGG
	R: CCGCCTTTGGGGGTAAATTC
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
β -Actin	F: GCTACAGCTTCACCACCACAG
	R: GGTCTTTACGGATGTCAACGTC
Note: F, forward; R, reverse; miR-145-5p, microRNA-145-5p; TGF- β 1, transforming growth factor β 1; ETS2, E26 transformation-specific proto-oncogene 2; Smad2/3, small mothers against decapentaplegic protein 2/3.	

Dual luciferase reporter gene assay

The 3'untranslated region (UTR) fragment of ETS2 was amplified by PCR and inserted into the vector. Using the Rapid Change Site-Directed Mutagenesis Kit (Agilent), the binding region of miR-145-5p in ETS2 3'UTR was mutated. Lipofectamine 2000 (Invitrogen) was employed to transfect ETS2 Wt 3'UTR or ETS2

Mut 3'UTR with mimic-NC or miR-145-5p mimic into HEK293T cells. The luciferase activity was analyzed by the dual luciferase reporter detection system (Promega, MI, USA) [27].

RNA immunoprecipitation (RIP) assay and RNA-pull down assay

Te RIP detection was performed using the Magna RIP RNA-binding protein immunoprecipitation kit (Millipore). Cells were lysed in RIP lysis buffer and then mixed with RIP buffer containing human anti-Ago2 antibody (Abcam). After digestion by proteinase K, the purified RNA was extracted and analyzed by RT-qPCR.

RNA-pull down assay: 3 biotinylated miR-145-5p or 3 biotinylated miRNC were transfected into cells. Then, cells were lysed and complex was separated with streptavidin agarose beads. ETS2 expression was evaluated via RT-qPCR [28].

Statistical analysis

SPSS software was applied to statistical analysis. The data were expressed as mean $\pm$ standard deviation. Each experiment was repeated at least 3 times. The statistical significance was measured by Student's t-test or non-parametric test. $P < 0.05$ was statistically significant.

Results

Construction of ALI model

miR-145-5p expression is decreased in renal ischemia-reperfusion injury [29], and ETS2 is up-regulated in ARDS [14]. miR-145-5p, ETS2, TGF- β 1 and Smad2/3 levels, as well as the phosphorylated (p)-Smad2/3 in rats with ALI were detected by RT-qPCR and Western blot. It was found that LPS-treated rats presented decreased miR-145-5p and increased ETS2 levels, and activated TGF- β 1/Smad signaling pathway (Fig. 1A-C). H&E staining captured obvious inflammatory cell infiltration, hemorrhage, interstitial edema and hyperplasia around the bronchioles and interstitium in LPS-treated rats (Fig. 1D); Masson staining manifested diffuse fibrosis, cell infiltration, alveolar wall thickening, alveolar structure destruction in LPS-treated rats (Fig. 1E). Also, LPS-treated rats had increased W/D ratio (Fig. 1F). After TUNEL staining, it was obvious that the number of apoptotic cells was increased and the brown particles were more obvious in LPS-treated rats (Fig. 1G). Detection of ELISA highlighted that serum levels of IL-6 and TNF- α were high in LPS-treated rats (Fig. 1H). Thus, ALI model was constructed successfully in rats.

Overexpression of miR-145-5p attenuates ALI and inactivates TGF- β 1/Smad pathway; Loss of miR-145-5p worsens ALI and activates TGF- β 1/Smad pathway

With the aim to determine the role of miR-145-5p in ALI, Ad-miR-145-5p or Adas-miR-145-5p was injected into rats with ALI. As a result of Ad-miR-145-5p injection, miR-145-5p expression was enhanced, ETS2 expression was reduced and the TGF- β 1/Smad pathway was inactivated (Fig. 2A-C); in response to Adas-

miR-145-5p injection, reduced miR-145-5p, elevated ETS2 levels and activated TGF- β 1/Smad pathway were detected in rats with ALI (Fig. 3A-C). In rats having up-regulated miR-145-5p, inflammatory cell infiltration, hemorrhage, interstitial edema and hyperplasia in the lung tissue sections were relieved, and diffuse fibrosis was reduced, the alveolar wall was no longer thickened, and the damage to the alveolar structure was weakened (Fig. 2D-E). At the same time, the lung W/D ratio, apoptosis rate, and serum contents of IL-6 and TNF- α were decreased (Fig. 2F-H). On the other hand, in rats having down-regulated miR-145-5p, the pathological damage, W/D ratio, apoptosis and inflammatory response were further worsened (Fig. 3D-H). Briefly, overexpression of miR-145-5p attenuated ALI and inactivated TGF- β 1/Smad pathway, while loss of miR-145-5p elicited the opposite consequence.

miR-145-5p targets ETS2

To explore the relation between miR-145-5p and ETS2, Jefferson website (<https://cm.jefferson.edu/rna22/Precomputed/>) was adopted to search for the specific binding site between the two (Fig. 4A). In dual luciferase gene reporter test, no change of luciferase activity showed in ETS2 Mut 3 UTR after transfection with miR-145-5p mimic, but reduced luciferase activity was measured in ETS2 Wt 3 UTR after transfection with miR-145-5p mimic (Fig. 4B).

RIP method measured that ETS2 expression was augmented in the sample bound to the Ago2 antibody (Fig. 4C); RNA pull-down assay found that ETS2 was enriched in biotinylated miR-145-5p (Fig. 4D). Shortly, ETS2 was a target of miR-145-5p.

Excessive expression of ETS2 aggravates ALI

To investigate the impact of ETS2 on ALI, LPS-treated rats were injected with oe-ETS2. RT-qPCR and Western blot verified that oe-ETS2 caused ETS2 expression increased and TGF- β 1/Smad pathway activated (Fig. 5A-C). Furthermore, after overexpression of ETS2, LPS-treated rats exhibited serious inflammatory cell infiltration, hemorrhage, interstitial edema, hyperplasia, and alveolar tissue damage (Fig. 5D, E), higher W/D ratio (Fig. 5F), increased apoptosis rate (Fig. 5G), and promoted inflammatory reaction (Fig. 5H). In summary, up-regulation of ETS2 exacerbated ALI in rats.

Elevation of ETS2 decreases miR-145-5p-mediated protection on rats against ALI

Finally, rescue test was conducted to figure out the mechanism of miR-145-5p/ETS2 axis in ALI. LPS-treated rats were injected with Ad-miR-145-5p and oe-ETS2. Consequently, oe-ETS2 reversed the effect of Ad-miR-145-5p on ETS2, TGF- β 1, Smad2/3 and p-Smad2/3 expression (Fig. 6A-C). Moreover, Ad-miR-145-5p-mediated attenuation on pathological damage in the lung tissues, W/D ratio, apoptosis and inflammation was mitigated by overexpressing ETS2 (Fig. 6D-H). Evidently, elevation of ETS2 decreased miR-145-5p-mediated protection against ALI.

Discussion

ALI represents a serious heterogeneous pulmonary disorder with high mortality [30]. In a LPS-induced rat model, the involvement and performance of miR-145-5p were precisely illustrated from the downstream target ETS2, and TGF- β 1/Smad signaling pathway. In short, miR-145-5p inactivated TGF- β 1/Smad signaling pathway and protected against ALI through targeted-modification of ETS2.

At first, in the lung tissue of ALI rats, miR-145-5p expression was measured to down-regulate. Then, gain- and loss-of-function assays were performed in ALI rats, and the results described that overexpression of miR-145-5p protected ALI rats in the aspects of pathological damage, apoptosis and inflammatory insult. On the contrary, depletion of miR-145-5p augmented ALI to progress. According to an article made by Y L Yu *et al.* it is known that miR-145-5p expression drops in mice with ALI, and the increase of miR-145-5p could suppress the levels of pro-inflammatory factors and limit oxidative stress insult [13].

Fundamentally, miR-145-5p is insufficiently expressed in patients with coronary heart disease, and overexpressing miR-145-5p strongly restrains apoptosis, inflammatory and oxidative stress reactions of injured cardiac microvascular endothelial cells [31]. Exactly, miR-145 expression is abated in the lung tissue of LPS-treated mice, and promoting miR-145 expression could reduce inflammatory stress and increase the overall survival of mice with lung injury after sepsis [12]. Similarly, geniposide-based therapy to obstruct apoptosis and inflammation of LPS-treated PC12 cells is reported to be facilitated by increasing miR-145-5p expression [32]. Furthermore, in hypoxia-injured cardiomyocytes, miR-145-5p expression is found to reach a low level; and impaired inflammation and apoptosis are seen in cardiomyocytes after introduction of higher miR-145-5p [33]. Besides, miR-145-5p expression is suppressed in patients with chronic obstructive pulmonary disease, and up-regulating miR-145-5p could at least attenuate apoptosis and inflammation of injured human pulmonary microvascular endothelial cells [34]. More interestingly, during the experiments, it was also recognized that increasing miR-145-5p expression inactivated TGF- β 1/Smad signaling pathway, as suggested by reduced TGF- β 1 and Smad2/3 expression. Consistently, there is an article illustrating that elevating miR-145-5p expression in fibroblasts could reduce Smad2/3 expression to attenuate hypertrophic scar [35]. Overall, miR-145-5p exerts considerable functions to attenuate pathological injury, including but not limited to ALI.

To proceed, ETS2 was validated as a target of miR-145-5p which was up-regulated in ALI rats. Also, functional assays were performed on ETS2 to reveal that enhancing ETS2 expression activated TGF- β 1/Smad signaling pathway and further deteriorated ALI in rats. In combination, elevation of ETS2 rescued the protective effect of miR-145-5p on ALI rats. Notably, Jing Li *et al.* have discovered that ETS2 expression is heightened in heart failure [36]. Based on a paper related to ARDS, ETS2 is excessively expressed in mice with ARDS, and depletion of ETS2 serves to improve lung function and inhibit the production of inflammatory parameters [14]. Lately, ETS2 expression has been determined to enhance in mice with renal fibrosis which could promote epithelial-to-mesenchymal transition [37]. Mechanistically, atherogenic condition could induce ETS2 expression to increase in endothelial cells, and the enriched ETS2 augments the formation and destabilization of atherosclerotic lesion, while the silenced ETS2 shows therapeutic effects [38]. Evidently, inactivation of ETS2 acts beneficially to enhance the anti-inflammatory effect of linarin in kidney ischemia/reperfusion injury [39]. Activation of ETS2 is able to

promote neutrophil migration and inflammation, but depleting ETS2 ameliorates inflammatory disease and reduces pathology in neutrophilic dermatoses [40].

Conclusion

On the whole, our research has discovered that the level of miR-145-5p is down-regulated, that of ETS2 is up-regulated and the TGF- β 1/Smad signaling pathway is activated in LPS-induced ALI. Moreover, overexpression of miR-145-5p or loss of ETS2 inactivates the TGF- β 1/Smad signaling pathway and attenuates the inflammatory injury in the lung induced by LPS. This study has refreshed miR-145-5p-related mechanisms and may provide a therapeutic reference for ALI. However, there is a large demand to validate and develop the research results in the proceeding studies.

Abbreviations

ALI

Acute lung injury

ARDS

acute respiratory distress syndrome

MiRNAs

MicroRNAs

LPS

lipopolysaccharide

ETS2

E26 transformation-specific proto-oncogene 2

PF

pulmonary fibrosis

TGF- β

transforming growth factor β

SPF

specific pathogen-free

W/D

Weight/dry

H&E

Hematoxylin-eosin

ELISA

Enzyme-linked immunosorbent assay

IL

levels of interleukin

TNF- α

tumor necrosis factor- α

TUNEL

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling

RT-qPCR

Reverse transcription quantitative polymerase chain reaction

UTR

untranslated region

RIP

RNA immunoprecipitation

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Care and Use Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Conflict of interest

The authors declare that they have no conflicts of interest.

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Authors' contributions

Hongjun Wu contributed to study design; Liang Qiao contributed to manuscript editing; Rongxia Li and Yu Liu contributed to experimental studies; Shangang Hu contributed to data analysis.

All authors read and approved the final manuscript.

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Figures

Figure 1

Construction of ALI model. A. miR-145-5p, ETS2, TGF- β 1 and Smad2/3 mRNA expression measured by RT-qPCR; B-C. ETS2, TGF- β 1 and P-Smad2/3 protein expression measured by Western blot; D. HE staining images ($\times 100$); E. Masson staining images ($\times 100$); F. W/D ratio in the lung; G. Apoptosis in the lung detected by TUNEL staining; H. Serum levels of IL-6 and TNF- α measured by ELISA; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (n = 5); * P < 0.05 vs. the Control group.

Figure 2

Overexpression of miR-145-5p attenuates ALI and inactivates TGF- β 1/Smad pathway. A. miR-145-5p, ETS2, TGF- β 1 and Smad2/3 mRNA expression measured by RT-qPCR; B-C. ETS2, TGF- β 1 and P-Smad2/3 protein expression measured by Western blot; D. HE staining images ($\times 100$); E. Masson staining images ($\times 100$); F. W/D ratio in the lung; G. Apoptosis in the lung detected by TUNEL staining; H. Serum levels of IL-6 and TNF- α measured by ELISA; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (n = 5); * P < 0.05 vs. the LPS + Ad-GFP group.

Figure 3

Loss of miR-145-5p worsens ALI and activates TGF- β 1/Smad pathway. A. miR-145-5p, ETS2, TGF- β 1 and Smad2/3 mRNA expression measured by RT-qPCR; B-C. ETS2, TGF- β 1 and P-Smad2/3 protein expression measured by Western blot; D. HE staining images ($\times 100$); E. Masson staining images ($\times 100$); F. W/D ratio in the lung; G. Apoptosis in the lung detected by TUNEL staining; H. Serum levels of IL-6 and TNF- α measured by ELISA; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (n = 5); * P < 0.05 vs. the LPS + Adas-GFP group.

Figure 4

miR-145-5p targets ETS2. A. The binding site of miR-145-5p and ETS2 predicted by Jefferson; B. The targeting relationship verified by dual luciferase reporter gene detection; C. ETS2 expression in samples bound to Ago2 antibody detected by RIP; D. ETS2 expression in biotinylated miR-145-5p sample; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (repetition = 3).

Figure 5

Excessive expression of ETS2 aggravates ALI. A. ETS2, TGF- β 1 and Smad2/3 mRNA expression measured by RT-qPCR; B-C. ETS2, TGF- β 1 and P-Smad2/3 protein expression measured by Western blot; D. HE staining images ($\times 100$); E. Masson staining images ($\times 100$); F. W/D ratio in the lung; G. Apoptosis in the lung detected by TUNEL staining; H. Serum levels of IL-6 and TNF- α measured by ELISA; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (n = 5); * P < 0.05 vs. the LPS + oe-NC group.

Figure 6

Elevation of ETS2 decreases miR-145-5p-mediated protection on rats against ALI. A. ETS2, TGF- β 1 and Smad2/3 mRNA expression measured by RT-qPCR; B-C. ETS2, TGF- β 1 and P-Smad2/3 protein expression measured by Western blot; D. HE staining images ($\times 100$); E. Masson staining images ($\times 100$); F. W/D ratio in the lung; G. Apoptosis in the lung detected by TUNEL staining; H. Serum levels of IL-6 and TNF- α measured by ELISA; the data were all measurement data, and the values were shown by the mean $\pm$ standard deviation (n = 5); * P < 0.05 vs. the LPS + Ad-miR-145-5p + oe-NC.