Different microRNAs contribute to the protective effect of mesenchymal stem cell-derived microvesicles in LPS induced acute respiratory distress syndrome

Xingcai Zhang 1, Lifang Ye 2, Guojin Liang 1, Wan Tang 1, Lifeng Yao 1, Changshun Huang 1*

1 Department of Anesthesiology, Ningbo First Hospital, No. 59 Liuting Street, Haishu District, Ningbo 315010, Zhejiang, China
2 Department of Anesthesiology, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, Guangdong, China

Abstract

Objective(s): The present study aimed to determine whether bone marrow mesenchymal stem cell-derived microvesicles (MSC MVs) were effective in restoring lung tissue structure, and to assess the potential role of miRNAs in the pathogenesis and progression of acute respiratory distress syndrome (ARDS).

Materials and Methods: ARDS was induced by lipopolysaccharide in male C57BL/6 mice. The degree of lung injury was assessed by histological analysis, lung’s wet weight/body weight, and protein levels in the bronchoalveolar lavage fluid (BALF). Sequencing was performed on the BGISEQ-500 platform. Differentially expressed miRNAs (DEM) were screened with DEGseq software. The target genes of DEMs were predicted by iRNAhybrid, miRanda, and TargetScan.

Results: Compared with LPS-injured mice, MSC MVs reduced lung water and total protein levels in the BALF, demonstrating a protective effect. 52 miRNAs were differentially expressed following treatment with MSC MVs in ARDS mice. Among them, miR-532-5p, miR-223-3p, and miR-744-5p were significantly regulated. Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed the target genes were mainly located in the cell, organelle, and membrane. Furthermore, KEGG pathways such as ErbB, PI3K-Akt, Ras, MAPK, Toll, and Wnt signaling pathways were the most significant pathways enriched by the target genes.

Conclusion: MSC MVs treatment was involved in alleviating lung injury and promoting lung tissue repair by dysregulated miRNAs.

Introduction

The 2019 novel coronavirus (2019-nCoV) is still spreading rapidly around the world, and the main cause of death in patients remains acute respiratory distress syndrome (ARDS) (1). ARDS is a severe form of hypoxic respiratory failure, with acute diffuse lung injury, characterized by inflammation leading to increased pulmonary vascular permeability and loss of aerated lung tissue (2). Although a large number of studies have revealed the pathophysiological basis of ARDS, clinically available drugs for its treatment have not reduced mortality, long-term mechanical ventilation time, and ICU hospitalization time in ARDS patients (3). The treatment options for ARDS are still limited. The high mortality rate of ~40% in the United States reflects the lack of efficient medical countermeasures for ARDS (4, 5). Hence, current efforts aimed to develop an effective treatment, and promoting lung tissue repair was the key to improving survival in patients with ARDS.

Because of their immunomodulatory capabilities, mesenchymal stem cells (MSCs) have been widely studied in animal models and clinically. A recent phase Ia clinical trial of MSCs for the treatment of ARDS confirmed a considerable improvement in cell viability from 36% to 85% (6). Interestingly, instead of cell transplantation, MSCs provided beneficial effects through paracrine mechanisms, including secretion of extracellular microvesicles (MVs) (7). Compared to MSCs, MVs have several advantages for use as therapeutic agents. For example, they lack self-replicating capabilities and have no risk of ectopic differentiation, tumor formation, genetic instability, and cellular rejection (8). As a potential “cell-free” therapy, MSC MVs reduced inflammation, promoted healing, and improved organ function (7).

Almost all biological processes, including development, hemostasis, and inflammation, are regulated by miRNAs (2). Currently, miRNAs have been identified as potential therapeutic targets in many diseases such as liver disease, heart disease, and cancer (9-11). Studies have demonstrated that different miRNAs regulated inflammatory reactions and tissue repair mechanisms in ALI/ARDS, which was accompanied by an increase of pro-inflammatory cytokines and a decrease of anti-inflammatory molecules (12-14).

Growing evidence indicated that MSC MVs were involved in protection against lung injury. However, how MSC MVs regulate miRNAs and participate in the pathogenesis and development of ARDS remains unknown. Hence, a successful mouse ARDS model
was established in this study, and miRNA expression was profiled following treatment with MSC-MVs, with the aim to determine the potential therapeutic role of MSC MVs in regulating miRNAs in the pathogenesis and development of ARDS. The findings provided new insights into the therapeutic strategy and mechanism of ARDS.

**Materials and Methods**

**Mesenchymal stem cells**

MSCs were purchased from Sciencell (https://www.sciencellonline.com). Cells were routinely identified and met the standard of the International Society of Cellular Therapy. MSCs, with a total passage number of 8, were used in these experiments. MSC-MVs were obtained from the supernatant of MSCs as described previously (15).

**Isolation of MSC MVs**

MSCs were cultured in MSC medium (DMEM with 10% fetal bovine serum and penicillin/streptomycin) until confluence in P75 flasks and serum-starved for 48 hr in fresh conditioned medium (α-MEM with 0.5% BSA, 2 mmol/L L-Glutamine, and 1% penicillin/streptomycin). To isolate the MVs, the conditioned medium of MSCs was centrifuged at 3,000 rpm for 20 min to remove cellular debris, then at 100,000g (Beckman Coulter; https://www.beckmancoulter.com/wsrportal/wsr/) to pellet the MVs for 1 hr at 48 °C. The pellet was washed with phosphate-buffered saline (PBS) and submitted to the second ultracentrifugation. MSC MVs were resuspended according to the final cell count of MSCs after 48 hr of serum starvation (10 µL of MVs per 1 × 10⁶ cells) and stored at -80 °C until further use (15).

**Identification of MSC MVs**

The isolated MSC MVs were observed under a scanning electron microscope to capture their structure. MSC MVs were fixed with 3% (w/v) Karnovsky fixative for 2 hr at 48 °C. The monolayers were post-fixed for 2 hr with 1% veronal buffered osmic acid and dehydrated in graded ethanol and/or propylene oxide. Cell preparations were then embedded in Epon or Araldite resin cured at 60 °C. Thin sections were contrasted with saturated aqueous uranyl acetate and Reynolds lead citrate. The sections were then imaged with a JEOL 1200 EX transmission electron microscope operating at 80 kV (16). MVs were also assessed for diameter distribution by flow cytometry. Moreover, MVs were evaluated by Western blot to identify specific markers such as CD63 and TSG101.

**ARDS induced by lipopolysaccharide in mice**

Male C57BL/6 mice (8-10 weeks old, n=6) were assessed in all experiments. The mice were purchased from Charles River. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Ningbo University. Mice were first anesthetized with chloral hydrate and LPS from *Escherichia coli* (Sigma) at 5 mg/kg (17). Mice were first anesthetized with chloral hydrate at 4% intraperitoneally. Different groups were given simultaneously while ARDS was initiated. The various groups included: (a) MSC MVs (100 µl), IT treatment group; (b) LPS (positive control), PBS (100 µl) treatment group; (c) control (negative control), no LPS induction or treatment, with operations similar to the other groups. After 24 hr, and in separate experiments, BALF samples and lungs were collected from mice to assess protein levels and histology, respectively.

**Lung wet weight/body weight and protein levels in BALF**

After the mice were euthanized and weighed, the lungs were extracted and weighed. BALF samples were obtained from mice at 24 hr after LPS-induced ARDS. Total protein concentration was measured in the BALF by the BCA method (BCA, lot: P0010, http://beyotime.com).

**Histology**

Lung samples from LPS-injured mice with or without treatment with MSC MVs were carefully excised at 24 hr and fixed with 4% paraformaldehyde. After fixation, lung samples were embedded in paraffin, cut into 5 µm sections, and stained with H&E. Meanwhile, part of the lung tissue excised was immediately stored in liquid nitrogen for further microRNA analysis.

**Identification of miRNAs**

Mice (n=3) were randomly selected from each group and sequenced on the BGISEQ-500 platform. With Bowtie (bowtiebio.sourceforge.net), cleaned miRNA sequencing reads were aligned to the human genome (GRCh38.p7 assembly) based on the Genome human UCSC reference annotation. With miRDeep2 (https://www.mdc-berlin.de/8551903/en/), the transcription abundance of miRNAs was determined. DEMs between the treatment and non-treatment groups were screened using the DEGseq software. The miRNA sequencing data generated in this paper is available in BioProject (PRJNA662018). For DEMs in the LPS+MV group versus LPS group, the threshold was defined as Q value<0.001 and |log2FC|>1.

**Identification of DEMs' target genes**

To improve the reliability of the predicted target genes, the target genes of DEMs were predicted by three algorithms, including iRNAhybrid (18), miRanda (19), and TargetScan (20), and filtered by the corresponding filter conditions such as free energy and score value. Genes that were simultaneously predicted by all three databases were identified as the target genes of DEMs.

**Functional annotation of DEMs**

Enrichment analysis was performed to assess whether a particular gene set was significantly enriched in a metabolic pathway, molecular function, or biological process. To further examine the biological functions of the target genes of DEMs, GO and KEGG pathway enrichment analyses of these DEMs between the LPS+MSC MVs and LPS groups were performed with the R package “hyper”. Adjusted P-values (Q values) for each pathway.
were obtained by FDR correction. Q value <0.05 was defined as the criterion for statistical significance.

**Statistical analysis**

Group pair comparisons were performed by unpaired t-test. For multiple group comparisons, ANOVA with Bonferroni correction was performed. P<0.05 was considered statistically significant. Analyses were performed with GraphPad Prism6 and Image. The severity of lung injury was semi-quantified with the lung injury score system by two investigators blinded to grouping.

**Results**

**Characterization of MSC MVs**

MSC MVs were characterized by electron microscopy, NanoSight analysis and Western blot, respectively. Scanning electron microscopy showed that the isolation technique yielded a homogeneous population of spheroid particles (Figure 1A). Then, Nanoflow detection showed that the diameters of MSC MVs were mostly around 67.72 nm, with a concentration of 1.16*10^{11} particles per ml (Figure 1B). The main protein composition of MSC MVs was analyzed by Western blot, which revealed that MSC MVs expressed membrane proteins commonly found in exosomes (CD63) and an endosome-associated protein (TSG101) (Figure 1C). These results confirmed that MVs derived from MSCs had the typical features of EVs in terms of size and protein marker profile.

**MSC MVs alleviated LPS-induced lung inflammation and injury**

To establish a successful model of ARDS, we chose 24 hr after vehicle or LPS instillation for assessment of lung inflammation and injury. Compared with the control group, there was increased alveolar septal thickening and elevated leukocyte infiltration in the alveolar and interstitial spaces in the LPS group. Compared with the LPS group, MSC MVs obviously relieved LPS-induced lung injury (Figure 2A). Lung injury scores were significantly increased in the LPS group compared with the LPS+MSC MVs group (Figure 2B). Pulmonary microvascular EC plays a pivotal role in the pulmonary inflammatory response. To address whether MSC MVs modulated pulmonary vascular EC barrier functions in vivo, BALF protein concentration and LWW/BW ratio were analyzed in LPS-induced ARDS mice. Total protein amounts were higher in the LPS group compared with the control group. Surprisingly, total protein concentrations were decreased in the LPS+MSC MVs group (Figure 2C). LWW/BW ratios (Figure 2D) in ARDS mice were significantly reduced by treatment with MSC MVs.

**Quality of the sequencing process**

SOAPnuke was used to control sample quality (https://github.com/BGI-flexlab/SOAPnuke). The base quality distribution reflected the accuracy of sequencing reads. The sequencer, sequencing reagents, and sample quality could all affect the quality of bases. The quality of the first few bases in the reads was not high, because random primers could not bind to the RNA template well during reverse transcription; as the length of sequencing increased, the ratio of high-quality bases...
increased. A low proportion of low-quality (Quality<20) bases indicated good sequencing quality (Figure 3).

**Identification of differentially expressed microRNAs**

Using a significance threshold of P<0.05, 72 miRNAs with differential expression between LPS and control groups were found, including 62 up-regulated and 10 down-regulated miRNAs. Among them, miR-146a-3p and miR-155-5p were significantly up-regulated (Figure 4A). Sequencing analysis revealed that 52 miRNAs were differentially expressed following treatment with MSC MVs in mice with ARDS. Of these 52 miRNAs, 49 were up-regulated and 3 were down-regulated. Among them, miR-532-5p, miR-223-3p, and miR-744-5p were significantly regulated (Figure 4C).

**Function and pathway analyses**

GO function and KEGG pathway analyses were performed to explore the biological functions of the identified target genes. Compared with the LPS group, genes were significantly enriched in cellular process, biological regulation, and metabolic process; in addition, the target genes were mainly located in the cell, organelle, and membrane (Figure 5B). Furthermore, KEGG pathways such as the ErbB, PI3K-Akt, Ras, MAPK, Toll, and Wnt signaling pathways were the most significant pathways enriched by the target genes in the LPS+MSC MVs group (Figure 5A).

**miRNA-gene regulation network construction and analysis**

In order to investigate the functions of differentially expressed miRNAs, we used three databases, including miRanda, miRTarBase, and TargetScan, to identify the target genes of lung tissue that were significantly up-regulated or down-regulated. Then, the confirmed

---

**Figure 4.** Volcano plots of DEMs in different groups. (A) 72 miRNAs were differentially expressed in ARDS mice. (C) 52 miRNAs were differentially expressed following treatment with MSCs MV in ARDS mice. Red, blue, and gray dots represent up-regulated, down-regulated, and non-differentially expressed miRNAs, respectively.

**Figure 5.** Enriched GO terms and KEGG pathways of DEMs in the LPS with MSC MVs group. A. KEGG pathways, where the color and size of the circle indicate the significance level (Q value) and the number of target genes involved, respectively. The x-axis shows the enrichment ratio and the y-axis shows KEGG pathways. B. GO classification, where the x- and y-axes represent the number of genes and the functional classification of GO, respectively.

**Figure 6.** DEMiRNA-miRNA interaction network. Interaction network between significantly down-regulated DEMs and up-regulated DEMs in the LPS with MSC MVs group. Compared with the LPS group, miR532-5p and miR223-3p were up-regulated, while miR744-5p was down-regulated.
DEmiRNA-DEmRNA interactions were further used for building a miRNA-gene regulation network (Figure 6).

Discussion

Studies have investigated the potential role of miRNAs in the pathogenesis of acute lung injury (ALI)/ARDS. MiRNAs are a class of highly conserved, noncoding single-stranded RNA molecules of approximately 18–22 nucleotides (21). Almost all biological processes, including development, hemostasis, and inflammation, were regulated by miRNAs. Multiple reports that analyzed the expression of miRNAs in pulmonary diseases were based on preclinical studies. However, the underlying mechanisms are not fully understood.

In this work, we found that compared with the control group, 72 miRNAs were differentially expressed in ARDS mice, including 62 up-regulated and 10 down-regulated. Among them, miR-146a-3p and miR-155-5p were significantly up-regulated. Previous studies confirmed that down-regulation of microRNA-146a-3p attenuated lipopolysaccharide-induced acute lung injury via SIRT1 up-regulation and NF-κB pathway regulation (22). As a key mediator of septic lung injury, serum exosomes from ALI mice delivered miR-155 to macrophages, stimulated nuclear factor κB (NF-κB) activation, and induced the production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 (23).

In most applications, MSCs were captured in the lungs and disappeared after a few days, with only a small proportion recovered at the injury site after intravenous injection (24, 25). It has been proposed that the immunomodulatory and regenerative effects of mesenchymal stem/stromal cells (MSCs) were mainly mediated by soluble paracrine factors and MSC-derived extracellular vesicles (MSC EVs)(26). Recent studies suggested that MSC EVs might serve as a novel and cell-free alternative to whole-cell therapies. Extracellular vesicle (EV) is a general term for membrane surrounded vesicles released by cells. According to size and biogenetic mechanisms, EVs are typically classified into three subtypes, including exosomes (50–150 nm), MVs (100–1000 nm), and apoptotic bodies (500–5000 nm)(27). Since no specific exosome and microvesicle markers have been identified, vesicles can only be fractioned according to size and/or density, regardless of their origin (8).

Previous studies confirmed that MSC MVs transfer depolarized mitochondria to macrophages, increase macrophage bioenergetics, and modulate TLR signaling and cytokine release in macrophages (28). In vitro, MSC MVs enhanced the functional integrity of injured endothelial cells, decreased lung pathology injury, modulated cytoskeletal signaling in endothelial cells, and attenuated lung vascular permeability (29, 30). In vivo, MSC MVs increased alveolar fluid clearance, reduced protein permeability and inflammation, and increased antimicrobial effects in the human lung model of bacterial pneumonia (26). In this study, the therapeutic effects of MSC MVs were examined in an LPS-induced ARDS mouse model. Histopathological examination indicated that lung injury was less prominent in the ARDS group treated with MSC MVs compared with the ARDS group. Compared with the LPS group, MSC MVs obviously relieved LPS-induced lung injury. Lung injury scores were significantly increased in the LPS group compared with the LPS+MSC MVs group. Total protein levels in the LPS group were higher than control values. Surprisingly, total protein concentrations in the BALF were decreased in the MSC MVs treatment group. LWW/BW ratios in mice were significantly reduced by treatment with MSC MVs. These results fully confirmed that MSC MVs alleviated LPS-induced lung inflammation and injury. Meanwhile, our data revealed 52 miRNAs were differentially expressed following treatment with MSC MVs in ARDS mice. Of the 52 miRNAs, 49 were up-regulated and 3 were down-regulated. Among them, miR532-5p, miR223-3p, and miR744-5p were significantly regulated. Previous investigations performing miRNA profiling in various inflammatory settings revealed that miR-223 was among the top differentially expressed miRNAs; indeed, the expression of miR-223 in an inflammatory model of GBS-induced murine pneumonia was rapidly induced at very early time points (3 to 6 hr post-infection) (31). MiR223 levels were significantly decreased in patients with non-surviving sepsis compared with surviving sepsis cases (32). Exosomes from MSCs overexpressing miR-223-3p suppressed the production of pro-inflammatory factors and promoted the secretion of anti-inflammatory factors, and attenuated cerebral ischemia/reperfusion injury by inhibiting microglial M1 polarization mediated pro-inflammatory response (33). Overexpression of miR-532-5p represses the elevation of inducible NOS, IL-6, IL-1 β, TNF-α, and MCP-1 in LPS-exposed BV2 cells (34). MiR-532-5p could inhibit the IL-1β-induced NF-κB proinflammatory signaling pathway, which suggested that miR-532-5p might be a potential anti-inflammatory mediator attenuating the symptoms of asthma (35). These miRNAs were detected in the present study in the lung tissue, which indicates that our approach provides reliable information regarding the miRNA expression profile.

In order to determine the biological effects of the target genes identified, we performed GO and KEGG pathway analyses of these genes in the lung after treatment with LPS or LPS with MSC MVs. The results showed the target genes were likely associated with ErbB, PI3K-Akt, Ras, MAPK, Toll, and Wnt signaling pathways. Both PI3K/Akt and MAPK intracellular cascades can be initiated by LPS-TRL4 receptor activation, leading to gene transcription and proinflammatory cytokine production; in particular, p38 MAPK plays a significant role in lung inflammation and cell apoptosis and is activated by LPS (36-38). The Toll-like receptor (TLR)4 receptor complex, TLR4/MD-2, plays an important role in the inflammatory response against lipopolysaccharide, and modifications in surface TLR4 expression via overexpression of Ras-related protein Rab10 in macrophages exacerbates LPS-induced lung injury (39). Therapeutic manipulation of Wnt signaling in endogenous stem cells may be exploited for tissue renewal and regeneration during early ARDS(40). GO classification demonstrated that the target genes were primarily related to cellular processes, biological regulation, and metabolic processes. Moreover, the target genes were mainly located in the cell, organelle, and membrane.

A single miRNA can have multiple target miRNAs,
and different sets of miRNAs are expressed in different cell types and tissues, with multiple functions in the development of many biological processes. In this study, a regulatory network of significantly up-regulated and down-regulated miRNA genes was built. However, the specific targeting relationships of these miRNA-mRNA sets need to be further verified.

This study had a few limitations. First, the small sample size might decrease the statistical power. In addition, the dysregulated miRNAs following LPS injection or MSC MVs infusion were not quantified by reverse transcription-quantitative polymerase chain reaction. Furthermore, we constructed a signaling pathway diagram based on sequencing results, but no further experiments were conducted to verify whether the effectors were involved in lung injury repair. Finally, MSC MVs were only administered via the tail vein to explore their protective effect on the lung. However, whether administration via the airway would be better remains unknown. Therefore, different treatment routes for MSC MVs deserved further investigation. In spite of these limitations, this study demonstrated that dysregulated miRNAs were involved in lung injury alleviation and lung tissue repair promotion in mice following treatment with MSC MVs.

Conclusion

In summary, MSC MVs attenuated lung injury via miRNA dysregulation, indicating the potential of miRNAs as therapeutic targets. Further studies are required to elucidate the putative targets of the identified dysregulated miRNAs.

Acknowledgment

This project was supported by the Medical and Health Plan of Zhejiang (grant no. 2019KY581). We would like to thank the Central Laboratory of Ningbo First Hospital, Zhejiang, China.

Authors’ Contributions

XCZ Conceived and designed research; XCZ and LFY Performed experiments; XCZ Wrote the manuscript; GJL Analyzed data; WT Interpreted results of experiments; LFY Prepared figures; CSH Edited and revised manuscript; CSH Approved final version of manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Stratton CW, Tang YW. Pathogenesis-directed therapy of 2019 novel coronavirus disease. J Med Virol 2021;93:1320-1342.
2. Ferruvelo A, Peñuelas O, Lorente JA. MicroRNAs as biomarkers of acute lung injury. Ann Transl Med 2018;6:34.
3. Bosma KJ, Tameja R, Lewis JF. Pharmacotherapy for prevention and treatment of acute respiratory distress syndrome: Current and experimental approaches. Drugs 2010;70:1255-1282.
4. Mäca J, Jor O, Holub M, Sklenka P, Burša F, Burda M, et al. Past and present ARDS mortality rates: A systematic review Respir Care 2017;62:113-122.
5. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, et al. Incidence and outcomes of acute lung injury. N Engl J Med 2005;353:1685-1693.
6. Mathy MA, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): A randomised phase 2a safety trial. Lancet Respir Med 2019;7:154-162.
7. Tieu A, Lalu MM. An analysis of mesenchymal stem cell-derived extracellular vesicles for preclinical use. ACS Nano 2020;14:9728-9743.
8. Jafarinia M, Alsahbehfoursei F. Mesenchymal stem cell-derived extracellular vesicles: A novel cell-free therapy. Immunol Invest 2020;49:758-780.
9. Bandiera S, Pfeiffer S, Baumert TF, Zeisel MB. miR-122—a key factor and therapeutic target in liver disease. J Hepatol 2015;62:448-457.
10. Wang JY, Zhang Q, Wang DD, Yan W, Sha HH, Zhao JH, et al. MiR-29a: A potential therapeutic target and promising biomarker in tumors. Biodis Rep 2018;38:20171265.
11. Bernardo BC, Ooi YJ, Lin RC, McMullen JR. miRNA therapeutics: A new class of drugs with potential therapeutic applications in the heart. Future Med Chem 2015;7:1771-1792.
12. Angulo M, Lecuna E, Snajder JI. Role of microRNAs in lung disease. Arch Bronconeumol 2012;48:325-330.
13. Mokra D, Kosutova P. Biomarkers in acute lung injury. Respir Physiol Neurobiol 2015;209:52-58.
14. Benz F, Roy S, Trautwein C, Roderburg C, Luedde T. Circulating microRNAs as biomarkers for sepsis. Int J Mol Sci 2016;17:78.
15. Hu S, Park J, Liu A, Lee J, Zhang X, Hao Q, et al. Mesenchymal stem cell microvesicles restore protein permeability across primary cultures of injured human lung microvascular endothelial cells. Stem Cells Transl Med 2018;7:615-624.
16. Tang XD, Shi L, Monsel A, Li XY, Zhu HL, Zhu YG, et al. Mesenchymal stem cell microvesicles attenuate acute lung injury in mice partly mediated by Ang-1 mRNA. Stem Cells 2017;35:1849-1859.
17. Wei W, Ma B, Li HY, Jia Y, Lv K, Wang G, et al. Biphasic effects of selective inhibition of transforming growth factor beta1 activin receptor-like kinase on LPS-induced lung injury. Shock 2010;33:218-224.
18. Krüger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. Nucleic Acids Res 2006;34:451-454.
19. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. PLoS Biol 2002;4:263.
20. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian miRNAs. Elife 2015;4:e05005.
21. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 2019;20:21-37.
22. Yang Y, Li L. Depleting microRNA-146a-3p attenuates lipopolysaccharide-induced acute lung injury via up-regulating SIRT1 and mediating NF-κB pathway. J Drug Target 2021;29:420-429.
23. Jiang K, Yang J, Guo S, Zhao G, Wu H, Deng G. Peripheral circulating exosome-mediated delivery of mir-155 as a novel mechanism for acute lung inflammation. Mol Ther 2019;27:1758-1771.
24. Katsha AM, Ohkouchi S, Xin H, Kanehira M, Sun R, Nukiwa T, et al. Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. Mol Ther 2011;19:196-203.
25. Tomi C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells for moderate to severe acute respiratory distress syndrome in the microvasculature: In vivo observations of cell kinetics. Circ Res 2009;104:399-402.
26. Park J, Kim S, Lim H, Liu A, Hu S, Lee J, et al. Therapeutic effects of human mesenchymal stem cell microvesicles in an ex vivo perfused human lung injured with severe Escherichia coli pneumonia. Thorax 2019;74:43-50.
27. Qiu G, Zheng G, Ge M, Wang J, Huang R, Shu Q, et al. Functional proteins of mesenchymal stem cell-derived extracellular vesicles. Stem Cell Res Ther 2019;10:359.
28. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun 2015;6:8472.
29. Shah T, Qin S, Vashi M, Predescu DN, Jeganathan N, Bardita C, et al. Alk5/Runx1 signaling mediated by extracellular vesicles promotes vascular repair in acute respiratory distress syndrome. Clin Transl Med 2018;7:19.
30. Potter DR, Miyazawa BY, Gibb SL, Deng X, Togaratti PP, Croze RH, et al. Mesenchymal stem cell-derived extracellular vesicles attenuate pulmonary vascular permeability and lung injury induced by hemorrhagic shock and trauma. J Trauma Acute Care Surg 2018;84:245-256.
31. Deny M, Romano M, Denis O, Casimir G, Chamekh M. Progressive control of streptococcus agalactiae-induced innate inflammatory response is associated with time course expression of MicroRNA-223 by neutrophils. Infect Immun 2020;88:563-520.
32. Wang H, Zhang P, Chen W, Feng D, Jia Y, Xie L. Serum microRNA signatures identified by Solexa sequencing predict sepsis patients’ mortality: A prospective observational study. PloSOne 2012;7:e38885.
33. Zhao Y, Gan Y, Xu G, Hua K, Liu D. Exosomes from MSCs overexpressing microRNA-223-3p attenuate cerebral ischemia through inhibiting microglial M1 polarization mediated inflammation. Life Sci 2020;260:118403.
34. Yan X, Zeng D, Zhu H, Zhang Y, Shi Y, Wu Y, et al. MiRNA-532-5p regulates CUMS-Induced depression-like behaviors and modulates LPS-Induced proinflammatory cytokine signaling by targeting STAT3. Neuropsychiatr Dis Treat 2020;16:2753-2764.
35. Li J, Panganiban R, Kho AT, McGechie MJ. Circulating MicroRNAs and treatment response in childhood asthma. Am J Respir Crit Care Med 2020;202:65-72.
36. Qiu X, Jiang X, Jiang X, Wang Y, Miao Z, He W, et al. Micheliolide inhibits LPS-induced inflammatory response and protects mice from LPS challenge. Sci Rep 2016;6:23240.
37. Wang X, Quinn PJ, Yan A. Kdo2-lipid A: Structural diversity and impact on immunopharmacology. Biol Rev Camb Philos Soc 2015;90:408-427.
38. Bode JG, Ehlting C, Häussinger D. The macrophage response towards LPS and its control through the p38(MAPK)-STAT3 axis. Cell Signal 2012;24:1185-1194.
39. Wang D, Lou J, Ouyang C, Chen W, Liu Y, Liu X, et al. Ras-related protein Rab10 facilitates TLR4 signaling by promoting replenishment of TLR4 onto the plasma membrane. Proceedings of the National Academy of Sciences of the United States of America. Proc Natl Acad Sci U S A 2010;107:13806-13811.
40. Villar J, Zhang H, Slutsky AS. Lung repair and regeneration in ARDS: Role of PECAM1 and Wnt signaling. Chest 2019;155:587-594.