Pulmonary MicroRNA Expression Profiling in an Immature Piglet Model of Cardiopulmonary Bypass-Induced Acute Lung Injury

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Abstract: After surgery performed under cardiopulmonary bypass (CPB), severe lung injury often occurs in infants. MicroRNAs (miRNAs) are potentially involved in diverse pathophysiological processes via regulation of gene expression. The objective of this study was to investigate differentially expressed miRNAs and their potential target genes in immature piglet lungs in response to CPB. Fourteen piglets aged 18.6 ± 0.5 days were equally divided into two groups that underwent sham sternotomy or CPB. The duration of aortic cross-clamping was 2 h, followed by 2 h reperfusion. Lung injury was evaluated by lung function indices, levels of cytokines, and histological changes. We applied miRNA microarray and quantitative real-time polymerase chain reaction (qRT-PCR) analysis to determine miRNA expression. Meanwhile, qRT-PCR and enzyme-linked immunosorbent assay were used for validation of predicted mRNA targets. The deterioration of lung function and histopathological changes revealed the piglets’ lungs were greatly impaired due to CPB. The levels of tumor necrosis factor alpha, interleukin 6, and interleukin 10 increased in the lung tissue after CPB. Using miRNA microarray, statistically significant differences were found in the levels of 16 miRNAs in the CPB group. Up-regulation of miR-21 was verified by PCR. We also observed down-regulation in the levels of miR-127, miR-145, and miR-204, which were correlated with increases in the expression of the products of their potential target genes PIK3CG, PTGS2, ACE, and IL6R in the CPB group, suggesting a potential role for miRNA in the regulation of inflammatory response. Our results show that CPB induces severe lung injury and dynamic changes in miRNA expression in piglet lungs. Moreover, the changes in miRNA levels and target gene expression may provide a basis for understanding the pathogenesis of CPB-induced injury to immature lungs. Key Words: MicroRNAs—Cardiopulmonary bypass—Acute lung injury—Inflammation—Real-time polymerase chain reaction.
regions or coding sequences and thereby suppressing target gene expression (6,7). Many recent studies have demonstrated that miRNAs play a role in regulating lung and systemic inflammation (8,9).

Previous studies examined the changes in miRNA expression in lung tissue using a murine model of lung injury induced by inhaled lipopolysaccharide or high-tidal-volume ventilation (HVTV) (10,11). In contrast, at present, there is a gap in large-animal studies of lung injury that simulate the clinical practice of cardiac surgery; such studies play an important role in translational research. To our knowledge, our study is the first report to address pulmonary miRNA changes in a clinically relevant CPB model. The purpose of this study was to measure miRNA profiling in ALI induced by CPB in a piglet model and to identify miRNA target genes with computational approaches while providing candidate miRNAs in the pathogenesis of CPB-induced ALI for further study.

MATERIALS AND METHODS

Animal care

All animal experimental procedures were approved in advance by the Committee on the Ethics of Animal Experiments of Fuwai Hospital and the Beijing Council on Animal Care, Beijing, China. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). All reasonable efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Experimental protocol

Fourteen healthy piglets of both genders, aged from 18 to 19 days (18.6 ± 0.5 days) and weighing 3.6 to 7.0 kg (5.1 ± 1.0 kg), were randomly divided into two groups of seven, each with equal gender distribution (four females and three males). In the CPB group (n = 7), all the piglets received 150 min CPB with 2 h of cardiac arrest and 2 h monitoring after CPB. Animals not submitted to CPB underwent only sternotomy for 270 min (control group, n = 7). Physiologic measurements were recorded, and lung tissue was harvested from the right lower lung lobe in both groups and stored in liquid nitrogen for further examination at the end of the study.

Anesthesia, operation, and CPB

To induce anesthesia, all the piglets were premedicated with intramuscular ketamine (10 mg/kg body weight [BW]) and given atropine (0.05 mg/kg BW) and midazolam (0.5 mg/kg BW) intravenously. Tracheal intubation was performed, and volume-controlled ventilation was used at a frequency of 25 to 35 breaths per minute, a tidal volume of 8 mL/kg BW, and a positive end-expiratory pressure of 4 cm H2O. Intravenous infusions of fentanyl (10 to 15 μg/kg BW/h) and vecuronium (0.1 to 0.12 mg/kg BW/h) were used to maintain anesthesia. In addition, all the piglets inhaled 1% sevoflurane from an anesthesia machine (Dräger Primus; Dräger, Lübeck, Germany). Fluid-filled catheters were placed into the right carotid artery for mean arterial pressure monitoring and blood gas analysis. Central venous pressure monitoring and medication administration were carried out through catheterization of the jugular vein. During the surgery, ECG and nasopharyngeal temperature were also monitored. Appropriate treatment was administered based on the indicators from monitor data.

A nonpulsatile systemic CPB flow was established at 2.4 L/min·m² via a 10 Fr aortic cannula and a 22 Fr venous cannula in the right atrial appendage after median sternotomy and heparinization (400–500 IU/kg [4–5 mg/kg] BW). The CPB device was equipped with a roller blood pump (Stöckert, Munich, Germany) and a CAPIOX RX05 Baby-RX Oxygenator (Terumo, Tokyo, Japan). A new and sterile CPB circuit (Tianjin Plastics Research Institute, Tianjin, China) was primed with 400 mL of donor porcine blood, 200 mL 6% hydroxyethyl starch, and heparin (400 IU/kg BW). Blood gas management was conducted according to the alpha-stat method.

When the piglets’ nasopharyngeal temperature was cooled to 32°C, cardiac arrest was obtained by aortic cross-clamp and antegrade infusion of St. Thomas’ cardioplegic solution at 4°C through the aortic root. After 120 min of cardiac arrest, piglets underwent assisted circulation and modified ultrafiltration and were rewarmed to a nasopharyngeal temperature of 35°C in about 30 min before weaning from CPB. Afterward, the piglets were given standard ventilation for 120 min before being sacrificed by intravenous hyperkalemic injection. Piglets unable to wean from CPB were excluded and replaced.

Evaluation of lung injury

The evaluation of lung injury was assessed by measuring lung function, cytokine concentration, and histology of pulmonary tissue. Lung compliance (LC)
and peak airway pressure (PAP) were obtained from the anesthesia machine. Alveolar–arterial oxygen gradient (P(A−a)O₂) was calculated based on arterial blood gas analysis (Nova Biomedical, Waltham, MA, USA) using the standard formula. Concentrations of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 10 (IL-10) in lung tissue were measured in duplicate with commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN, USA).

The lungs of a random sample from each group were inflated by intratracheal instillation of 4% paraformaldehyde solution at a hydrostatic pressure of 18 cm H₂O. The lungs were then fixed in 4% paraformaldehyde for 24 h and embedded in paraffin. Sections of 4 μm were stained with hematoxylin and eosin for light-microscope observation. For each paraffin section sample, 20 random fields were examined at 400× magnification, and lung injury in each field was scored by two pathologists, who were blinded to the groups according to the recommendations of the American Thoracic Society (12).

miRNA microarray analysis and gene target prediction for miRNAs

Total RNA was isolated from lung tissue by using the mirVana RNA Isolation Kit (Applied Biosystems p/n AM1556; Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. The quantity and quality of RNA were measured using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The total RNA was analyzed by microarray (Affymetrix miRNA 2.0; Affymetrix, Santa Clara, CA, USA) provided by a service provider (Shanghai Biotechnology Corporation, Shanghai, China), and sequences were obtained from the Sanger miRBase, release 17.0.

In brief, after being 3′-extended with a poly(A) tail using poly(A) polymerase, the 3DNA dendrimer was ligated to total RNA to allow multiple biotins (~15) to bind to each poly(A)-tailed RNA molecule. Following FlashTag ligation (Affymetrix), samples were hybridized on the microarray overnight. After washing and staining, the hybridization signals were detected by an Affymetrix scanner. Scanner images were quantified by the Affymetrix GeneChip Convert Console software. The hybridization data were normalized with the miRNA QC tool software using the default parameters and analyzed with GeneSpring 10 (Silicon Genetics, Redwood City, CA, USA). The data were analyzed with Student’s t-test, and P values < 0.05 were identified as indicating differentially expressed genes.

*Sus scrofa* miRNA mature sequences and mRNA sequences were downloaded from the miRBase database and NCBI RefSeq, respectively (13). Gene target prediction was mainly performed by querying the miRNA Database miRanda (14) with the following parameters: gap open penalty −9.0, gap extend penalty −4.0, score threshold 80.0, energy threshold −20.0 kcal/mol, scaling parameter 2.0 (15).

Real-time PCR validation of miRNA and target gene expression

For miRNA expression detection, reverse transcriptase and real-time quantitative polymerase chain reaction (qPCR) of miRNA genes were assessed with the Qiagen miRNA qPCR detection kit (GeneCopoeia, Inc., Rockville, MD, USA).

Each reverse transcription reaction contained 2 μg RNA, 2.5 U poly(A) polymerase, 1 μL RTase mix, and 1× reaction buffer in a final volume of 25 μL. The reaction mixtures were incubated at 37°C for 60 min, then at 85°C for 5 min, and then diluted five times with RNase-free water. Reverse transcriptase reactions, including no-template controls and RT-minus controls, were run in duplicate.

Then, real-time qPCR was conducted with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Real-time PCR was performed using the miScript SYBR Green PCR kit (Qiagen, Venlo, The Netherlands). For each reaction, 2 μL diluted first-strand cDNA was mixed with 10 μL 2× QuantiTect SYBR Green PCR Master Mix (Qiagen), 0.4 μL 50× ROX reference dye (Life Technologies, Carlsbad, CA, USA), and 0.2 μM forward and reverse primer in a final volume of 20 μL. The amplification conditions were as follows: 40 cycles at 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s. The reverse primer was the universal reverse adaptor PCR primer, while the forward primers were synthesized mainly according to the mature sequences of the tested miRNAs. The miRNA validation qPCR reactions and sequences of forward primers used in this study are listed in Table 1.

For mRNA expression detection, 2 μg of total RNA was reverse-transcribed with Moloney murine leukemia virus transcriptase (Superscript II, Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. qRT-PCR was performed with the One-Step Plus system (Applied Biosystems). The primer sequences of target genes are listed in Table 2. Typically, each sample is done in triplicate.
The relative expression level was calculated using the \(2^{(-\Delta \Delta CT)}\) method (16); levels of each miRNA and mRNA were calculated by threshold cycle (CT) and normalized to the expressions of small RNU6B and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively (17,18).

### Measurement of target gene expression in lung tissue

Frozen lung tissue was homogenized in normal saline and centrifuged to obtain supernatant for measurement. The concentrations of protein products of target genes in lung tissue were quantified in duplicate with ELISA kits (Fanke Biotech Co., Ltd., Shanghai, China) according to the manufacturer’s instructions.

### Statistical analysis

All values are given as mean \(\pm\) standard deviation (SD). Data were analyzed with SPSS 18.0 software for Windows (IBM, Armonk, NY, USA). Differences between groups were tested for significance by Student’s \(t\)-test for unpaired samples. A value of \(P < 0.05\) was considered statistically significant.

### RESULTS

#### Lung injury induced by CPB

When lung function indices were considered, \(P_{(A-a)O_2}\) and PAP increased and LC decreased significantly in the CPB group. Meanwhile, the levels of TNF-\(\alpha\), IL-6, and IL-10 were significantly higher in lung tissue from the CPB group compared to that from the control group (\(P < 0.05\)) (Table 3).

Histological evaluation of lung tissue in the CPB group showed increased neutrophil infiltration in the interstitial and alveolar space and more proteinaceous debris filling the airspaces (Fig. 1). We based lung injury scoring on five parameters: (1) neutrophils in the alveolar space, (2) neutrophils in the interstitial space, (3) hyaline membranes, (4) proteinaceous debris filling the airspaces, and (5) alveolar septal thickening. Each of the five histological parameters was graded using a three-tiered schema, with final score being determined by the

### TABLE 1. Primer sequences used for qRT-PCR analysis of miRNAs and predicted target genes

<table>
<thead>
<tr>
<th>Assay name</th>
<th>miRBase access number</th>
<th>Primer sequence</th>
<th>Predicted miRNA target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssc-miR-127</td>
<td>MI0013144</td>
<td>TCGGATCCGTCTGAGCTTGG</td>
<td>TIF2, PIK3CG, IFNGR1</td>
</tr>
<tr>
<td>ssc-miR-145</td>
<td>MI0002417</td>
<td>GTCCAGTTTTTCCCAGGAATCC</td>
<td>ACE, TNC, IL6R</td>
</tr>
<tr>
<td>ssc-miR-204</td>
<td>MI0002458</td>
<td>TTCCCTTTGTCACTCCTATGCCT</td>
<td>MMP25, PTGS2, F5</td>
</tr>
<tr>
<td>ssc-miR-21</td>
<td>MI0002459</td>
<td>TAGCTTATCAGACTGATGTTGA</td>
<td>ERMP1</td>
</tr>
<tr>
<td>Human-RNU6B*</td>
<td>NR_002752</td>
<td>TTCGTGAAGCCTTCATATTTT</td>
<td>—</td>
</tr>
</tbody>
</table>

*Control.

### TABLE 2. The nucleotide sequences of primers for the validation of miRNA targets

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward primer (5' to 3')</th>
<th>Reverse primer (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-PIK3CG</td>
<td>AATGTTCAGTGTAGGCTTTATTTGC</td>
<td>TCAGAAGCCTACGTACCAAGAAAG</td>
</tr>
<tr>
<td>S-ACE</td>
<td>GACTGGCCTTGGACGAGGAA</td>
<td>TTCCAGGCAAGAAGTGTGAC</td>
</tr>
<tr>
<td>S-IL6R</td>
<td>GGTACACATTGCCCACTTCCC</td>
<td>AAGATGATGCAATGCATAGGA</td>
</tr>
<tr>
<td>S-MMP25</td>
<td>CGTGGAAATTAACCGTGCTACATT</td>
<td>ATCCAGGAATTAGATTCTACAACACCT</td>
</tr>
<tr>
<td>S-PTGS2</td>
<td>CTTTCTGCTGAAAGCCCATTCG</td>
<td>ATCTGGGGAGCGCTTCTCTA</td>
</tr>
<tr>
<td>sus-GAPDH</td>
<td>TGCCGCCTGGAGAAACCT</td>
<td>GCAATCAAGAGTGAAGTGTAGTG</td>
</tr>
</tbody>
</table>

### TABLE 3. Evaluation of lung injury

<table>
<thead>
<tr>
<th>Lung function indices</th>
<th>Control</th>
<th>CPB</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP (cm H(_2)O)</td>
<td>8.20 ± 1.79</td>
<td>27.00 ± 3.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(P_{(A-a)O_2}) (mm Hg)</td>
<td>215.11 ± 15.10</td>
<td>468.35 ± 61.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LC</td>
<td>8.20 ± 1.21</td>
<td>2.02 ± 0.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Levels of inflammatory cytokines in lung tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/mL)</td>
<td>21.56 ± 9.33</td>
<td>36.08 ± 6.48</td>
<td>0.021</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>133.03 ± 26.28</td>
<td>175.47 ± 29.58</td>
<td>0.043</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>17.56 ± 1.02</td>
<td>20.67 ± 1.45</td>
<td>0.004</td>
</tr>
</tbody>
</table>
equation as mentioned (12). Lung injury scores of piglets treated with CPB were more severe (CPB $0.69 \pm 0.03$, control $0.08 \pm 0.01$; $P < 0.05$).

### Changes in lung miRNA expression profile induced by CPB

In the array examination and differential analyses of 189 miRNAs, levels of 16 miRNAs were found to be significantly changed in lung tissue in CPB animals; nine miRNAs were up-regulated, whereas seven miRNAs were down-regulated (Table 4).

### Confirmation of the expression of miRNAs by qRT-PCR analysis

To verify the accuracy of the microarray results above, we selected 4 of the 16 differentially expressed miRNAs for validation because they had a relatively lower $P$ value or a previously reported association with inflammatory response. We noted that the miRBase database showed that miR-1308 was not a miRNA but a 5′-cleaved fragment of a tRNA (GlyGCC). Given the lack of a normalizer miRNA, quantification of its expression was not performed. qRT-PCR verified the data for the four differentially expressed miRNAs, except miR-204, which was found to be down-regulated in the CPB group (Fig. 2).

### Expression levels of miRNA target genes

To identify the potential targets for differentially expressed miRNAs, we screened their sequences against the *Sus scrofa* database and used the miRNA target identification programs miRBase

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**TABLE 4.** MicroRNAs differentially expressed in lung tissue of piglets that received CPB as determined by microarray

<table>
<thead>
<tr>
<th>miRNA</th>
<th>CPB/control (signal)</th>
<th>Fold change</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssc-let-7f</td>
<td>11.02/10.25</td>
<td>1.68</td>
<td>0.0473</td>
</tr>
<tr>
<td>ssc-let-7g</td>
<td>11.56/10.87</td>
<td>1.59</td>
<td>0.0458</td>
</tr>
<tr>
<td>ssc-let-7i</td>
<td>8.51/7.69</td>
<td>1.76</td>
<td>0.0018</td>
</tr>
<tr>
<td>ssc-miR-127</td>
<td>6.20/7.08</td>
<td>0.54</td>
<td>0.0163</td>
</tr>
<tr>
<td>ssc-miR-1308</td>
<td>12.37/10.91</td>
<td>2.88</td>
<td>0.0417</td>
</tr>
<tr>
<td>ssc-miR-145</td>
<td>13.61/13.76</td>
<td>0.90</td>
<td>0.0494</td>
</tr>
<tr>
<td>ssc-miR-151-5p</td>
<td>12.19/12.31</td>
<td>0.92</td>
<td>0.0398</td>
</tr>
<tr>
<td>ssc-miR-181a</td>
<td>12.49/12.76</td>
<td>0.83</td>
<td>0.0485</td>
</tr>
<tr>
<td>ssc-miR-199a-3p</td>
<td>12.71/12.41</td>
<td>1.23</td>
<td>0.0193</td>
</tr>
<tr>
<td>ssc-miR-204</td>
<td>6.46/5.79</td>
<td>1.60</td>
<td>0.005593</td>
</tr>
<tr>
<td>ssc-miR-208b</td>
<td>4.01/3.89</td>
<td>1.09</td>
<td>0.047005</td>
</tr>
<tr>
<td>ssc-miR-21</td>
<td>9.77/8.44</td>
<td>2.57</td>
<td>0.02271</td>
</tr>
<tr>
<td>ssc-miR-331-3p</td>
<td>7.51/7.18</td>
<td>1.26</td>
<td>0.011422</td>
</tr>
<tr>
<td>ssc-miR-339</td>
<td>9.09/9.68</td>
<td>0.67</td>
<td>0.046699</td>
</tr>
<tr>
<td>ssc-miR-425-3p</td>
<td>7.01/7.55</td>
<td>0.69</td>
<td>0.04045</td>
</tr>
<tr>
<td>ssc-miR-935</td>
<td>3.79/4.14</td>
<td>0.78</td>
<td>0.044386</td>
</tr>
</tbody>
</table>

---

**FIG. 1.** Histology of lungs in control group (A) and CPB group (B). CPB-induced neutrophil infiltration (arrows), alveolar septal thickening (asterisks), and hyaline membranes (arrowhead). Magnification 400×. Scale bar = 50 μm. Hematoxylin–eosin stain.

**FIG. 2.** The changes in miRNA expression levels in CPB-induced ALI were analyzed by qRT-PCR. *$P < 0.05$ versus control.
and miRanda as described above (Table 1). Then, we measured pulmonary levels of mRNAs for phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG), angiotensin-converting enzyme (ACE), interleukin 6 receptor (IL6R), matrix metalloproteinase 25 (MMP25), and prostaglandin-endoperoxide synthase 2 (PTGS2), which are targeted by miRNAs with inflammation-associated target genes and closely related to lung injury.

As shown in Fig. 3, we found that as miR-127 levels decreased, the level of PIK3CG mRNA was up-regulated \((P < 0.05)\). Pulmonary PTGS2 mRNA levels increased after CPB \((P < 0.05)\), consistent with the decrease in miR-204 expression, whereas there was no significant difference in the expression of MMP25 mRNA compared with the control group. ACE and IL6R mRNA levels in lung increased after CPB \((P < 0.05)\), consistent with the decrease in miR-145 expression. Furthermore, the ELISA results also revealed levels of the protein products of the miRNA target genes to be elevated, except MMP25, which was consistent with PCR validation (Fig. 4).

**DISCUSSION**

miRNAs are emerging as important regulators of inflammatory and IR injury. Previous reports have shown that miRNAs are involved in HVTV-related lung injury (11) and cardiac and renal IR injury (19). However, whether miRNAs are involved in CPB-related ALI has not been described in previous research.

In this study, we performed CPB in ten piglets, of which seven completed the study successfully. Two piglets were excluded due to major bleeding during the surgical procedure, and one because of sudden cardiac arrest during assisted circulation after the aorta was unclamped. The piglet model simulated clinical practice successfully: CPB resulted in lung dysfunction, as evidenced by increased PAP and
PTGS2, also called cyclooxygenase 2 (COX-2), whose coding gene is a putative target gene of miR-204, is known to account for the release of large quantities of proinflammatory prostaglandins at the site of inflammation (28). Evidence also suggests that the COX-2 protein plays an important role in the pathogenesis of ARDS. COX-2 is normally undetected in most tissue; it is up-regulated in response to a variety of proinflammatory stimuli, such as TNF-α and interleukins (29,30).

The phosphatidylinositol 3-kinases (PI3K), whose coding genes are potential targets of miR-127, are a family of proteins that catalyze phosphorylation of phosphoinositides, generating lipid second messengers that control a wide variety of intracellular signaling pathways involved in a broad array of cellular responses including survival, proliferation, and activation (31). Yum et al. found that PI3K plays a central role in regulating neutrophil activation in endotoxin-induced lung injury (32).

Angiotensin II is a significant proinflammatory mediator that induces the production of reactive oxygen species and inflammatory cytokines; it is mediated by ACE, whose coding gene is a putative target of miR-145, in the renin–angiotensin system. It is reported that ventilation of healthy animals results in an inflammatory response in the lung, which is mediated by ACE via an increased production of its effector peptide, angiotensin II (33). Meanwhile, IL-6 exerts its biological activities through IL6R, whose coding gene is the target of miR-145, which thus plays a critical role in IL-6 signaling, which contributes to the development of inflammation and lung injury (34,35).

MMP25, whose coding gene is another target of miR-204, may contribute to cytokine secretion and respiratory burst in polymorphonuclear neutrophils (36). However, we did not find any differential expression in MMP25 between the two groups in the present study. The level of MMP25 might be down-regulated by other miRNAs in the CPB group; further validation in vitro is needed.

On the whole, changes in expression of the target genes provided evidence that CPB stimulated inflammatory response in the lungs of the piglets. Most importantly, as far as we are concerned, reestablishing expression of the anti-inflammatory miRNAs miR-204, miR-127, and miR-145 and diminishing expression of the pro-inflammatory miRNA miR-21 may be explored as a potential new treatment for CPB-related lung injury.

The limitations of the present study have to be taken into account when considering the interpretation of our results. This study was performed in a
short-term animal model with a short postbypass time and limited measurements. Due to the low body weight of the piglets used in our study, it was not possible to collect lung tissue samples sequentially over several time points with safety. As we chose the piglet as the animal model, there were only 189 miRNAs examined in microarray analysis. This smaller sample size may also have decreased the sensitivity of our analysis to unknown miRNAs that may have participated in the CPB-related ALI mechanism. Last but not least, we had difficulty finding a suitable miRNA database for Sus scrofa for gene target prediction. Meanwhile, current articles about miRNA in lung injury cannot be applied directly in CPB models.

As the identification of the molecular target and cellular source of each altered miRNA in vitro was beyond the range of our study, the mechanisms by which miRNAs affected lung injury in CPB were not fully understood. It is possible that not all differentially expressed miRNAs contribute to the pathogenesis of CPB-related ALI.

**CONCLUSION**

This study showed that dynamic changes occurred in microRNA expression in piglet lungs in cardiopulmonary bypass-related acute lung injury. Higher miR-21 level and lower levels of miR-204, miR-127, and miR-145 correlated to severe acute lung injury and poor lung function. We have identified candidate miRNAs that may be critical modulators in pathogenesis of CPB-related ALI and may provide a new possibility for lung protection in CHD surgery, which warrants further studies.

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**Author contributions:** Wenlei Li did experiments, analyzed data and drafted the article. Kai Ma and Sen Zhang did experiments and data analysis. Hao Zhang designed and supervised experiments and performed critical revision of the article. Jinping Liu designed and performed experiments. Xu Wang provided advice about experiments and data analysis; Shoujun Li designed the study, performed critical revision of the article, and obtained funding.

**Conflict of Interest:** The authors declare that there are no conflicts of interest.

**REFERENCES**


