CircRNA expression profiling and bioinformatics analysis indicate the potential biological role and clinical significance of circRNA in influenza A virus-induced lung injury

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Circular RNA (circRNA) plays an important role in the regulation of multiple biological processes. However, circRNA profiling and the potential biological role of circRNA in influenza A virus (IAV)-induced lung injury have not been investigated. In the present study, circRNA expression profiles in lung tissues from mice with and without IAV-induced lung injury were analyzed using high-throughput sequencing, and differentially expressed circRNAs were verified by quantitative PCR. The gene homology of candidate circRNAs was investigated and the expression of plasma circRNAs from patients with IAV-induced acute respiratory distress syndrome (ARDS) was detected. The target microRNAs (miRNAs) of circRNAs were predicted. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed. In total, 781 circRNAs were differentially expressed between ARDS mice and control (467 were up-regulated and 314 were down-regulated). Moreover, the candidate circRNAs (Slco3a1, Nfatc2, Wdr33, and Dmd) expression showed the same trend with the sequencing results. The isoforms of circRNA Slco3a1 and Wdr33 were highly conserved between humans and mice. Plasma circRNA Slco3a1 and Wdr33 presented differential expression in patients with IAV-induced ARDS compared to control. The circRNA-miRNA interaction network and GO and KEGG analyses indicated the potential biological role of circRNAs in the development of IAV-induced lung injury. Taken together, a large number of differentially expressed circRNAs were identified in our study. CircRNA Slco3a1 and Wdr33 had significantly different expression in specimens from mice and humans, and showed a potential biological role in IAV-induced lung injury by bioinformatics analysis.

Keywords. circRNA Slco3a1; circRNA Wdr33; miRNA; influenza; lung injury

Abbreviations. ARDS, acute respiratory distress syndrome; circRNA, circular RNA; GO, gene ontology; IAV, influenza A virus; KEGG, Kyoto encyclopedia of genes and genomes; miRNA, MicroRNA; qPCR, quantitative PCR.

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1. Introduction

Acute respiratory distress syndrome (ARDS) is a common complication of influenza A virus (IAV) infection, making it a leading cause of death during influenza epidemic season. The general incidence of ARDS caused by IAV infection is about 2.7 cases per 100000 person-years and accounts for about 4% of hospitalizations due to respiratory failure in influenza epidemic periods (Ortiz et al. 2013). Our previous study indicated that ARDS is a leading cause of admission to the intensive care unit and the incidence of ARDS caused by H7N9 virus and H1N1 virus is 58.3% and 27.3%, respectively (Wang et al. 2017a). The hospitalization cost and mortality in these patients are significantly higher than those in non-ARDS patients with IAV infection.

The symptoms of ARDS are nonspecific, making it a challenge to distinguish from cardiac pulmonary edema and severe pneumonia. Although therapeutic strategies for ARDS have greatly improved during the past two decades, the general incidence of mortality in patients with moderate and severe ARDS is still more than 40% (Thompson et al. 2017). Thus, identifying biomarkers and clarifying the potential mechanism of IAV-induced ARDS could contribute to an early diagnosis, better treatment, and prognostic accuracy.

Circular RNA (circRNA) is a novel type of RNA characterized by covalently closed continuous loops. Gaining a better understanding of the biological roles of circRNA, such as microRNA (miRNA) sponges, RNA-binding protein sequestering agents, and nuclear transcriptional regulators, will provide additional insights into the cellular physiology and disease pathogenesis (Chen 2016). CircRNAs have potential applications as disease biomarkers and novel therapeutic targets in cardiovascular disease, neurological disease, and cancer (Han et al. 2018). However, comprehensive circRNA profiling has not been performed in IAV-induced lung injury, and the underlying role of circRNAs is still unknown.

In this study, we investigated the expression profile of circRNA in lung tissues from IAV-infected mice through high-throughput RNA sequencing, and identified a large number of differentially expressed circRNAs between IAV group and control group. The expression of candidate circRNAs was detected in specimens from mice and humans, and bioinformatics analysis was also performed. The results indicate that circRNAs play a potential role in IAV-induced lung injury.

2. Materials and methods

2.1 Establishment of the mouse model

A/PR/8/34(PR8), a mouse-adapted H1N1 virus strain, has been commonly used for the development of mouse model of influenza A virus-induced lung injury. Here, we used this virus strain as a representative of influenza A virus. Specific pathogen-free male 6-week-old C57B/L6 mice, obtained from the Laboratory Animal Center of Soochow University (Suzhou, China), were anesthetized with 10% chloral hydrate in saline (0.1 mL/10 g) by intraperitoneal injection. Then the mice were challenged intranasally with 15 μL 10^5 pfu PR8 or phosphate-buffered saline for each nostril. Four days after challenge, the mice were sacrificed by cervical vertebral dislocation. The whole lungs were separated and harvested. The left lungs were fixed in 4% formaldehyde solution for 24 h and then embedded in paraffin. The 5 mm thick sections were stained with hematoxylin and eosin (H&E) and used for lung injury analysis under a microscope. The right lungs were snap frozen in liquid nitrogen and stored at −80°C for high-throughput RNA sequencing and validation of candidate circRNAs.

2.2 High-throughput RNA sequencing of circRNA

Total RNA was isolated using the Hipure Total RNA Mini Kit (Magen, Guangzhou, China) according to the protocol. The concentration and integrity of the total RNA were evaluated by the Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and Agilent 2100 Bioanalyzer (Applied Biosystems, Carlsbad, CA, USA), respectively. RNA samples with an RNA integrity number value of at least 7.0 or higher were used for further processing. The RNA sequencing library was prepared with approximately 2 μg total RNA using the KAPA RNA HyperPrep Kit with RiboErase for Illumina® (Kapa Biosystems, Inc., Woburn, MA, USA). Briefly, ribosomal RNA was removed from the total RNA. The ribosome-depleted RNA was incubated at 37°C for 30 min with 10 units RNase R (Epicentre Technologies, Madison, WI, USA) and purified with VAHTS RNA Clean Beads. Next, ribominus RNase R (+) RNAs were fragmented and then first-strand and directional second-strand synthesis were performed. Then A tailing and adapter ligation were performed with the purified cDNA. Finally, the purified, adapter-ligated DNA was amplified. The library quality and concentration were assessed by
utilizing the DNA 1000 chip on the Agilent 2100 Bioanalyzer. Accurate quantification of sequencing applications was determined using the quantitative PCR (qPCR)-based KAPA Biosystems Library Quantification Kit (Kapa Biosystems). Each library was diluted to a final concentration of 10 nM and pooled equimolar prior to clustering. Paired-End (PE150) sequencing was performed on all samples.

2.3 Analysis of differentially expressed circRNAs

Differentially expressed circRNAs between the IAV group and control group were screened by using the “edgeR” package in R. Fold change greater than 2.0 and p value less than 0.05 were considered statistically significant for differentially expressed circRNAs. A volcano plot with clustering for the differentially expressed circRNAs was constructed with the “gplots” package in R.

2.4 Detection of candidate circRNAs

Six circRNAs in mouse lung tissues from the top 20 differentially expressed circRNAs were selected for further validation using qPCR in 10 specimens (5 specimens in each group), and the predicted human conserved circRNAs were also tested in human plasma (5 specimens for each group). The total RNA was extracted with TRIzol® Reagent (Invitrogen) and reverse-transcribed to synthesize first-strand cDNA with the Geneseed® II First Strand cDNA Synthesis Kit (Geneseed, Guangzhou, China). Then qPCR was performed with the Applied Biosystems 7500 Real-Time PCR system. The housekeeping genes β-actin and U6 were used as controls and the relative expression of circRNAs was calculated with the $2^{-\Delta\Delta CT}$ method. The specific primers for these candidate circRNAs were designed (Table 1).

2.5 Prediction of human conserved circRNAs

LiftOver tool (http://genome.ucsc.edu/cgi-bin/hgLiftOver) was used to identify human conserved circRNAs (parameters:-bedPlus = 3-tab-minMatch = 0.1-minBlocks = 1). We first investigated the back-spooling site of a circRNA and obtained the corresponding location in the mouse genome. If the corresponding human genome was located at the back-spooling site of the circRNA in the circbase database, the circRNA was considered conservative, and then the ID number of the circbase was made available. The homology comparison between mice and humans was

| Genes name | circbase ID | Primers | Sequences | Product length (bp) |
|------------|-------------|---------|-----------|---------------------|
| Slco3a1    | mmu_circ_0014392 | Forward  | 5'-CAGCCACGAACATGATGTACT-3' | 149 |
|            |              | Reverse  | 5'-GTCAACACGCTACCTATGTTGTATG-3' | 118 |
|            | hsa_circ_0003252 | Forward  | 5'-CTCGCTCTATATAAGTCTAGGACG-3' | 118 |
|            |              | Reverse  | 5'-AGAGGATGAGCAGAAGGTGGTTC-3' | 118 |
| Fam53b     | mmu_circ_0013952 | Forward  | 5'-GCCTAGATGACAGAAATGAA-3' | 173 |
|            |              | Reverse  | 5'-GTCTTTGATTAGGATCTGC-3' | 173 |
| Sept9      | mmu_circ_0002788 | Forward  | 5'-AGCTGAGGCACACAACTGTC-3' | 143 |
|            |              | Reverse  | 5'-ACGATCTCTTAAACGGCTTC-3' | 143 |
| Nfatc2     | mmu_circ_0009613 | Forward  | 5'-CCTCTGTGTCAGAAGACCTC-3' | 148 |
|            |              | Reverse  | 5'-AAAGCCTTGCCAGCAAGATG-3' | 148 |
| Wdr33      | mmu_circ_0008585 | Forward  | 5'-GGAGAAAGGATTCTCCAGTCTGACT-3' | 172 |
|            |              | Reverse  | 5'-ATCAAGGAGGTAGCTGATCTGATT-3' | 172 |
|            | hsa_circ_0003192 | Forward  | 5'-AGTCTTCTACCCACGGATA-3' | 116 |
|            |              | Reverse  | 5'-TTCCTCTGATAGAACTCCG-3' | 116 |
| Dmd        | mmu_circ_0016387 | Forward  | 5'-CAGATAGATGTCAGCCTATG-3' | 165 |
|            |              | Reverse  | 5'-GAGCAGGAGGAGGCTTCTTGAA-3' | 165 |
| β-actin    | Forward  | 5'-GCTTTCTAGGACGCTTAC-3' | 100 |
|            | Reverse  | 5'-CCATGCAAATGTGTGCTTAC-3' | 100 |
| U6         | Forward  | 5'-CTCGTCTTCAGGACGACCACA-3' | 94 |
|            | Reverse  | 5'-AACGCTTCACGAAATTTCGTT-3' | 94 |

Table 1. Primer sequences for each gene.
performed with the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6 Prediction of target miRNAs for candidate circRNAs and the functional analysis

The miRanda databases were used to identify the possible binding of miRNAs with circRNAs. The main criteria for predicting a possible circRNA-miRNA interaction including sequence matching, thermal stability (free energy) of double-stranded binding of miRNA-circRNA, and conservativeness of target sites. The predicted miRNAs with a total score $> 140$ and total energy $<-20$ were enrolled in our study. All miRNA gene targets were identified with a $p < 0.05$. The networks between circRNAs and miRNAs were built with Cytoscape and the miRNAs with the top 10 total scores were enrolled in the networks. Gene Ontology (GO) analysis (http://geneontology.org/) was performed to predict the potential function of circRNAs in terms of biological process, cellular components, and molecular functions. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (http://www.genome.jp/kegg/) was also performed to further explore the signaling pathways of circRNA-related target genes. The significance threshold was classified as $p < 0.05$ and FDR value $< 0.01$.

2.7 Collection of patient plasma and ethical statement

Patients with IAV-induced ARDS and paired healthy individuals were enrolled in our study. Peripheral blood from all participants (6 mL) was collected in an EDTA tube and stored at 4°C for less than 1 h. The blood should be centrifuged within 1 h at $3000 \times g$ for 15 min to obtain plasma, and then stored at $-80°C$. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Soochow University, and informed consents were obtained from all participants.

2.8 Statistical analysis

All values were presented as the mean ± standard deviation. The difference between two groups was evaluated by the independent samples $t$-test. All of the statistical analyses were performed using SPSS 22.0 software package and visualized by Graphpad Prism 5.0. A $p < 0.05$ was considered statistically significant.

3. Results

3.1 CircRNA expression profiling and the distribution of differentially expressed circRNAs in mouse chromosomes

Pathological features of lung tissues from IAV-infected mice were characterized by serious alveolar collapse and consolidation, significant alveolar congestion and hemorrhage, and extensive inflammatory cell infiltration (figure 1A). Lung tissue from the control group indicated normal lung histology (figure 1B). Compared to the control group, circRNA expression in lung tissue from IAV-infected mice presented a greater than two-fold change and $p < 0.05$ were considered biologically meaningful. In total, 781 differentially expressed circRNAs were identified, of which 467 were significantly up-regulated and 314 were down-regulated (figure 1C). Read statistics (Table S1) and read quality plot (supplementary figure 1) of next-generation sequencing have been given as supplementary data. Volcano plots were used to visualize differentially expressed circRNAs between the two groups (figure 1D). Furthermore, the top 20 up-regulated and 20 down-regulated circRNAs were listed according to fold change in expression (table 2). We also investigated the distribution of differentially expressed circRNAs in mouse chromosomes. The circular plot indicated differentially expressed circRNAs located in almost all mouse chromosomes, except chromosome Y (supplementary figure 2A). The circRNAs were mostly located on chromosomes 2 and 5 (64 for each chromosome). The up-regulated circRNAs were mostly located on chromosome 5 and the down-regulated circRNAs were mostly located on chromosomes 2 and 9 (supplementary figure 2B).

3.2 Validation of candidate circRNAs and the gene homology analysis of differentially expressed circRNAs

Four up-regulated (circRNA Slco3a1, Fam53b, Sept9, and Nfatc2) and two down-regulated (circRNA Wdr33 and Dmd) circRNAs from the table 2 were selected for further analysis by qPCR in 10 lung tissues (5 samples for each group). The results showed that circRNA Slco3a1 and circRNA Nfatc2 were significantly up-regulated in lung tissues from IAV-infected mice compared with the control group, and circRNA Wdr33
and circRNA Dmd were significantly down-regulated (figure 2A). Among the 781 differentially expressed circRNAs in mice, 323 were confirmed to be conserved in humans (41.36%). Furthermore, we found 192 human conserved circRNAs in 467 up-regulated circRNAs (41.11%) and 131 human conserved circRNAs in 314 down-regulated circRNAs (41.72%) (figure 2B). Specifically, we found that the circRNA Slco3a1 isoform located on chr7_74504176_74504642 in the mouse genome (mmu_circ_0014392) and chr15_92459222_92459688 in the human genome (hsa_circ_0003252) were highly conserved with identities of 88% (supplementary figure 3A). CircRNA Wdr33 isoform located on chr18_31827261_31835481 in the mouse genome (mmu_circ_0000858) and chr2_128520635_128528578 in the human genome (hsa_circ_0003192) were highly conserved with identities of 93% (supplementary figure 3B).

3.3 CircRNA-miRNA interaction networks and the expression level of human plasma circRNA Slco3a1 and circRNA Wdr33

We constructed circRNA-miRNA interaction networks for all differentially expressed circRNAs.
supplementary figure 4). We also constructed circRNA-miRNA interaction networks for circRNA Slco3a1 and Wdr33. The 10 highest ranking target miRNAs for each circRNA isoform were enrolled in the networks (figure 3A–D). The relative expression of plasma circRNA Slco3a1 and circRNA Wdr33 in ARDS patients was quantified with qPCR technology. CircRNA Slco3a1 was significantly up-regulated and circRNA Wdr33 was significantly down-regulated in ARDS patients compared to control (figure 3E, F).

| Gene         | mmu_circbase_id | Fold change | Difference | P value |
|--------------|----------------|-------------|------------|---------|
| Nab1         | mmu_circ_0008772 | 28.856      | up         | 0.000   |
| Ambra1       | mmu_circ_0001046 | 12.245      | up         | 0.002   |
| Slco3a1      | mmu_circ_0014392 | 9.205       | up         | 0.000   |
| Zfp516       | mmu_circ_0007569 | 8.909       | up         | 0.036   |
| Fam53b       | mmu_circ_0013952 | 7.454       | up         | 0.000   |
| Rbp4         | mmu_circ_0011224 | 6.825       | up         | 0.008   |
| Copb1        | mmu_circ_0001615 | 6.75        | up         | 0.019   |
| Vps54        | mmu_circ_0002874 | 6.658       | up         | 0.003   |
| Galnt7       | mmu_circ_0014869 | 6.475       | up         | 0.023   |
| Zkscan1      | mmu_circ_0012225 | 6.348       | up         | 0.014   |
| Cry11        | mmu_circ_0000543 | 6.229       | up         | 0.029   |
| Usp12        | mmu_circ_0012340 | 6.185       | up         | 0.003   |
| Lats2        | mmu_circ_0005309 | 5.894       | up         | 0.000   |
| Nfat2        | mmu_circ_0001103 | 5.528       | up         | 0.000   |
| 4933426M11Rik| mmu_circ_0004228 | 5.228       | up         | 0.02    |
| Sept9        | mmu_circ_0002788 | 5.045       | up         | 0.001   |
| Sle10a7      | mmu_circ_0014994 | 4.977       | up         | 0.024   |
| Nfatc2       | mmu_circ_0009613 | 4.847       | up         | 0.000   |
| Lphn3        | mmu_circ_0012809 | 4.598       | up         | 0.028   |
| Tor1a        | mmu_circ_0009821 | 4.567       | up         | 0.011   |
| Mlpl         | mmu_circ_0001809 | -33.767     | down       | 0.000   |
| Hace1        | mmu_circ_0002239 | -16.502     | down       | 0.002   |
| Ptp1a        | mmu_circ_0009382 | -13.949     | down       | 0.001   |
| Ptk2         | mmu_circ_0005767 | -12.103     | down       | 0.015   |
| Poc1b        | mmu_circ_0002616 | -11.664     | down       | 0.001   |
| Tbc             | mmu_circ_0002854   | -9.439       | down       | 0.043   |
| Gdap2        | mmu_circ_0010973 | -8.643      | down       | 0.048   |
| Wdr33        | mmu_circ_0000858 | -8.243      | down       | 0.004   |
| Asc2         | mmu_circ_0003047 | -7.558      | down       | 0.001   |
| Akap7        | mmu_circ_0000153 | -6.783      | down       | 0.013   |
| Gli3         | mmu_circ_0004514 | -6.707      | down       | 0.048   |
| Bmpr1b       | mmu_circ_0010499 | -5.9436     | down       | 0.034   |
| Atf7ip       | mmu_circ_0013135 | -5.336      | down       | 0.017   |
| Pcm1         | mmu_circ_0002393 | -5.089      | down       | 0.035   |
| Arid2        | mmu_circ_0006009 | -4.794      | down       | 0.01    |
| Dmd          | mmu_circ_0016387 | -4.576      | down       | 0.024   |
| Argap32      | mmu_circ_0001764 | -3.604      | down       | 0.000   |
| Cdk8         | mmu_circ_0012324 | -3.553      | down       | 0.004   |
| Boc          | mmu_circ_0006305 | -3.36       | down       | 0.001   |
| Gfra1        | mmu_circ_0007972 | -3.287      | down       | 0.001   |

3.4 GO and KEGG analyses of circRNA Slco3a1 and Wdr33 in mice and humans

Biological process analysis demonstrated that the isoforms of circRNA Slco3a1 and Wdr33 in mice and humans were mainly involved in synthesis and metabolism of biomolecules, mitochondrial function, and superoxide metabolic process (figures 4A, 5A). Cellular components analysis of circRNA Slco3a1 and Wdr33 showed their target genes were mainly implicated in mitochondria and/or Golgi apparatus.
Molecular functions analysis of these two circRNAs showed their target genes were mainly enriched in terms of enzyme activity regulation such as cofactor binding, coenzyme binding, and peptidase regulator activity (figures 4C, 5C). KEGG analysis revealed that the main pathways related to the isoforms of circRNA Slco3a1 and Wdr33 in mice were carbon metabolism, hippo signaling pathway, and oxidative phosphorylation (figure 4D). However, the signaling pathways of the isoforms of circRNA Slco3a1 and Wdr33 in humans were mainly associated with pathogen infection, the c-type lectin receptor signaling pathway, and notch signaling pathway (figure 5D).

4. Discussion

In the present study, we investigated circRNA profiling in mice with IAV-induced lung injury using high-throughput RNA sequencing, and found abundant differentially expressed circRNAs. The differential expression of candidate circRNAs was validated in mice lung tissues, and also confirmed in plasma from ARDS patients with IAV infection by qPCR technology. The interaction networks between circRNAs and their target miRNAs were constructed. In addition, GO and KEGG analyses were also performed, from which the potential biological role and clinical significance of circRNAs in the development of IAV-induced lung injury were preliminarily demonstrated.

Circular RNAs, a novel class of noncoding RNAs characterized by covalently closed continuous loops without 5'–3' polarity and a polyadenylated tail, play an important role in the genetic regulation of multiple biological process (Salzman 2016). They have features of high abundance and stability, are evolutionarily conserved among species, and are expressed in a tissue-specific manner and pathology-specific context, indicating their potential applications as suitable biomarkers and novel therapeutic targets (Qu et al. 2015).

ARDS is a common complication in clinical settings and is also a life-threatening form of respiratory failure in critically ill patients. ARDS most often develops in the context of pneumonia (such as bacterial and viral) and sepsis (Matthay et al. 2019). Since the high morbidity and mortality remain major challenges in the management of ARDS patients, basic research on ARDS development would be beneficial for preventing or treating this disease (Fan et al. 2018). To date, little is known about the role of circRNAs in the pathophysiological process of ARDS. In a previous study, the expression profiles of circRNAs were analyzed in lung tissues from rats with LPS-induced ARDS and a large number of differentially expressed circRNAs was found (Wan et al. 2017). In another study, comprehensive circular RNA profiling in LPS-induced acute lung injury mice preliminary demonstrated aberrantly expressed circRNAs and the potential molecular mechanism (Li et al. 2019b). In the current study, we first investigated the expression profiles of circRNAs in mice with IAV-induced lung injury and found a large number of circRNAs expressed in a pathology-specific manner.
Approximately 5–30% of circRNAs are completely conserved in closely related species such as humans and mice. In our study, we found 41.36% of human conserved circRNAs in differentially expressed circRNAs in mice. As two circRNA isoforms of gene Slco3a1 and Wdr33, mmu_circ_0014392 and hsa_circ_0003252, mmu_circ_0000858 and hsa_circ_0003192 were highly conserved. CircRNA Slco3a1 and circRNA Wdr33 were aberrantly expressed in the lung tissues of the ARDS animal model and plasma from ARDS patients, indicating the potential role of circRNA Slco3a1 and circRNA Wdr33 in the development of IAV-induced ARDS.

It has been reported that circRNAs can regulate gene expression through the regulation of transcription and alternative splicing, interaction with RNA-binding proteins, miRNA sponge, and even translation (Han et al. 2018). Among these mechanism of genetic regulation, the functions of acting as miRNA sponges or competitive endogenous RNAs have garnered great attention (Rossbach 2019). In this study, the circRNA-miRNA interaction network was established and a possibly extensive interaction between circRNAs and miRNAs was predicted. After reviewing the literature on these target miRNAs, we found that some are reportedly involved in virus infection.
and/or inflammation regulation. As predicted, the target miRNA of circRNA Slco3a, miRNA-15b, is involved in Japanese encephalitis virus infection and can alleviate high glucose-induced cell apoptosis, oxidative stress, and inflammatory responses (Zhu et al. 2016; Fu et al. 2019; Shen et al. 2019). It has been reported that miRNA-16 can alleviate lipopolysaccharide (LPS)-induced A549 cell injury, inhibit NLRP3 inflammasome activation, and suppress enterovirus 71 replication (Zheng et al. 2017; Liu et al. 2019; Yang et al. 2019). As target miRNAs of circRNA Wdr33, miRNA-543 and miRNA-130b are reportedly involved in the regulation of ameliorating inflammatory injury in multiple pathophysiological processes (Wang et al. 2017b; Zheng et al. 2018; Zhao et al. 2019). To further investigate the biological role of those target genes contained in the circRNA-miRNA network, we also performed GO and KEGG analyses. Biological process analysis revealed that circRNA Slco3a1 and Wdr33 might be involved in mitochondrial function and superoxide metabolic process. Superoxide and mitochondrial dysfunction are associated with inflammatory response, alveolar epithelial cells apoptosis, and lung barrier impairment (Liu et al. 2014; Song et al. 2016). Furthermore, KEGG analysis showed that signaling pathways, such as the hippo signaling pathway, oxidative phosphorylation, and notch signaling pathway, had a close relationship with lung injury (Li et al. 2015, 2019a, 2020). These bioinformatics analyses provide insights into the vital regulatory role of circRNAs Slco3a1 and Wdr33 in the development of IAV-induced lung injury. It is notable that KEGG analysis revealed different signaling pathways in mice and humans, indicating the complex regulatory mechanism of circRNA in different species. It has been confirmed that circRNA GATAD2A can promote H1N1 virus replication by inhibiting autophagy. However, to date, comprehensive profiling and the potential role of circRNA in

Figure 4. Functional annotation of circRNA Slco3a1 and circRNA Wdr33 in mice. The columns listed in GO and KEGG analyses indicate the top 10 significant enrichment. (A) Biological process analysis; (B) Cellular components analysis; (C) Molecular functions analysis; (D) KEGG pathway analysis.
IAV-induced lung injury has not been explored (Yu et al. 2019).

In conclusion, this is the first study to explore the expression profile of circRNAs in IVA-induced lung injury. After the detection of candidate circRNAs in specimens from both mice and humans, gene homology analysis, construction of circRNAs and miRNAs interaction networks, and GO and KEGG analyses, we found the potential biological role and clinical significance of circRNAs in IAV-induced lung injury. Additional studies are needed to reveal the function and underlying mechanism of circRNA Slco3a1 and circRNA Wdr33 in IAV-induced lung injury.

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