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ORIGINAL ARTICLE

The differential expression of novel circular RNAs in an acute lung injury rat model caused by smoke inhalation

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Abstract Acute lung injury caused by smoke inhalation is a common severe clinical syndrome. This study aimed to investigate the potential expression of circular RNAs during acute lung injury triggered by smoke inhalation. The acute lung injury rat model was established with smoke inhalation from a self-made smoke generator. The occurrence of acute lung injury was validated by an analysis of the bronchoalveolar lavage fluid and hematoxylin-eosin (HE) staining of lung tissues. Next-generation sequencing and quantitative PCR were performed to identify the differentially expressed circular RNAs associated with acute lung injury that was caused by smoke inhalation. The circular form of the identified RNAs was finally verified by multiple RT-PCR-based assays. The bronchoalveolar lavage fluid (BALF) and lung tissue analysis showed that smoke inhalation successfully induced acute injury in rats, as evidenced by the significantly altered cell numbers, including macrophages, neutrophils, and red blood cells, disrupted cell lining, and increased levels of interleukin-1β, tumor necrosis factor-alpha, and IL-8 in lung tissues. Ten significantly differentially expressed circular RNAs were identified with next-generation sequencing and RT-PCR. The circular form of these RNAs was verified by multiple RT-PCR-based assays. In conclusion, the identified circular RNAs were prevalently and differentially expressed in rat lungs after acute lung injury caused by smoke inhalation.

Keywords Acute lung injury · Smoke inhalation · Circular RNA · Rat model

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Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) refer to the common acute respiratory failure associated with substantial morbidity and mortality worldwide. They usually feature lung endothelial and epithelial barrier disruption and could lead to impaired long-term quality of life, even for those who survive ALI [18, 20]. Patients with ALI commonly suffer from disrupted alveolar–capillary membrane integrity. This membrane is composed of the microvascular endothelium, interstitium, and alveolar epithelium, and its disruption consists of the release of cytotoxic and pro-inflammatory factors, as well as excessive trans-epithelial neutrophil migration [9]. ALI is caused by a number of factors, which could be categorized into direct injuries, including pneumonia, drowning, gastric aspiration, pulmonary contusion, fat and amniotic-fluid embolism, alveolar hemorrhage, toxic gas or smoke inhalation, reperfusion, and lung re-implantation. Indirect mechanisms of injury include severe sepsis, transfusions, shock, salicylate or narcotic overdose, and pancreatitis [20]. Burn injuries occur in large numbers of patients around the world, and the ensuing morbidity and mortality increase significantly when it is due to acute lung injury associated with smoke inhalation [5]. Smoke inhalation can greatly aggravate lung edema, a clinical condition referring to

the aberrant fluid flux from the circulating plasma to interstitial spaces caused by vascular hyperpermeability [16]. Although several mediating factors, such as nitric oxide overproduction, activation of poly(ADP-ribose) polymerase (PARP)-related signaling, and airway obstruction, were revealed in a previous investigation [5], the exact molecular mechanisms underlying acute lung injury associated with smoke inhalation remain unclear.

Circular RNAs (circRNAs) are a group of RNA molecules currently classified as part of the non-coding RNA (ncRNA) superfamily, which accounts for approximately 95% of total RNA in eukaryotic cells [2]. Circular RNAs are very different from linear RNAs and exist in covalently closed continuous loops by the ligation of the 3′ and 5′ ends, which has been shown to stem from the back-splicing of exons or introns [3, 6]. The first circular RNA model was identified in 1976, but at the time, was considered to be a useless product of RNA splicing errors [15]. Recent accidental discoveries have shown that various species of circular RNA molecules exist with high abundance, stability, and evolutionary conservation, and were subsequently found to play important roles in the regulation of gene expression, possibly as miRNA sponges partially mediated by the competitive endogenous RNA (ceRNA) network [8]. The expression of circRNAs is tissue-specific and has been verified in pathological conditions associated with various human diseases, such as ischemic heart disease, Alzheimer's disease, and diabetes mellitus, as well as different malignant tumors like gastric, colon, hepatocellular, and lung cancers [6]. Accumulating evidence has demonstrated the potential of circular RNAs as novel biomarkers for disease diagnosis and treatment, but the specific roles and underlying mechanisms in their regulation of gene expression remains to be clarified.

The expression of circRNAs in lung tissues has been demonstrated in various physiological and pathological contexts. The circular RNA-ITCH (cir-ITCH) was detected in both the tumor tissues and the adjacent non-cancerous tissues from 78 lung cancer patients, as well as in multiple lung cancer cell lines [19]. The expression of cir-ITCH was shown to be significantly suppressed in lung cancer tissues, and further investigation demonstrated that cir-ITCH regulated the expression of a cancer suppressor gene as an miR-7 and miR-214 sponge, thus repressing lung cancer proliferation [19]. Recently, another circular RNA, hsa_circ_0013958, was also identified as a novel potential biomarker for lung cancer [25]. More importantly, a number of circular RNAs were differently expressed in mouse lung tissues that had been exposed to radon, one of the most toxic environmental radioactive gases and a known carcinogen for lung cancer [14], suggesting a possible role of circular RNA as mediator during the acute response of lung tissue to external hazardous substances. However, the involvement of circRNAs in ALI, including that induced by smoke inhalation, has not previously been reported.

In order to address the possible function of circRNAs in the pathogenesis of ALI, a rat model of acute lung injury was established and the differentially expressed circRNAs during the progression of ALI induced by smoke inhalation in rat lung tissues were identified and confirmed in this study. These findings provide an important basis for research and future studies of circRNAs in ALI.

Material and methods

Animals and model construction

The 24 male Wistar rats (aged 8–10 weeks) used in this study were purchased from the SLAC Laboratory Animal Co., Ltd. (Shanghai, China). For the construction of the smoke inhalation-induced ALI model, the rats were randomly divided into three groups ($n = 8$ per group): the control, the 6-h ALI (6 h) group, and the 24-h ALI (24 h) group. Rats from the 6- and 24-h groups were subjected to smoke inhalation for 15 min in a self-made smoke generator after general anesthesia induced by intraperitoneal injection of 20 mg/kg sodium pentobarbital. After 6 or 24 h, the rats were sacrificed by intraperitoneal injection of an overdose of sodium pentobarbital (150 mg/kg body weight), and the bronchoalveolar lavage fluid (BALF) and lung tissues were collected. The control group was treated with the same protocol, except for the smoke inhalation. All experiments were authorized by the Animal Care and Ethics Committee of Sun Yat-sen University.

Bronchoalveolar lavage fluid and lung tissue collection

The bronchoalveolar lavage fluid (BALF) collection was carried out as previously described [11]. Briefly, after being treated with smoke exposure as indicated, the airways and lungs of rats were immediately subjected to a trachea cannula and lavage with Hank's balanced salts solution (0.6 mL, HBSS) purchased from Thermo Fisher Scientific Co., Ltd. (USA). Collected BALF was centrifuged at $4000 \times g$ and 4 °C for 15 min, and the supernatant was frozen at − 80 °C for the following analysis. The rat lung tissues were collected by professional surgeons following standard protocols.

BALF protein concentration and cell count

The concentration of proteins in the rat BALF was measured with a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific Inc., USA) according to the manufacturer's protocols. The counting of macrophages (MAC), neutrophils (NEU), and red blood cells (RBC) was finished using BALF smears with hematoxylin-eosin (HE) staining under microscopy.

Enzyme-linked immunosorbent assay

The protein levels of interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and IL-8 in the lung tissues of rats after smoke inhalation and the control group were determined by enzyme-linked immunosorbent assay using the corresponding kits according to the manufacturer's instructions. Specifically, the IL-1β content was analyzed with the Rat IL-1 β ELISA Kit ([#]RAB0277, Sigma-Aldrich, USA), the TNF-α content was analyzed with the Rat Tumor Necrosis Factor α ELISA Kit ([#]RAB0479, Sigma-Aldrich, USA), and the IL-8 content was determined using the Rat IL-8 (Interleukin 8) ELISA Kit ([#]E-EL-R0560, Elabscience, USA). For the statistical analysis, three biological repeats were carried out for the quantitation of each protein in the three different groups.

Circular RNA identification and quantitation with next-generation sequencing

The identification and quantitative analysis of differentially expressed circular RNAs between rat lung tissues of the 24-h group and those of the control group were performed as previously described [4, 22, 23]. Briefly, lung tissue samples were homogenized in liquid nitrogen for total RNA extraction using the Animal Tissue RNA Purification Kit ([#]25700, Norgen Biotek Corporation, USA) according to the manufacturer's instructions. The rRNA molecules were removed with the rRNA-binding magnetic bead method, and the linear RNAs were digested with RNase R ([#]RNR07250, Epicentre, USA). The remaining RNA samples were used for cDNA synthesis using random primers, then analyzed by the Agilent 2100 Bioanalyzer System, quantified by QPCR, and finally sequenced with an Illumina sequencer [23]. The raw sequencing data was checked for quality control, and the clean filtering reads were used for bioinformatic blasting against circBase reference sequences [13], as previously described [4]. The number of clean reads matched to reference sequences was recorded, followed by expression level quantitation and differentially expressed circular RNA identification. GO and pathway enrichment analysis were finally performed.

Quantitative PCR and RT-PCR

Next-generation sequencing was used to confirm the expression levels of ten circRNAs with differential expression between the lung tissues of the 24-h group and the control group with quantitative polymerase chain reaction (Q-PCR), as previously described [1] and were statistically analyzed. In order to verify the circular shape of the identified molecules, total RNA extraction and cDNA synthesis were performed as described above, and were then analyzed by PCR using the specific primers listed in Table 1. RT-PCR was also combined with sequencing or RNase digestion to further confirm the circular shape.

Statistical analysis

All the statistical analyses of significance differences in this study were performed using the SPSS software package (version 18.0, SPSS). Student's t test was used to analyze the significance of differences with three biological replicates. The significant and extremely significant differences were defined by a P value < 0.05 or < 0.01.

Results

Smoke inhalation alters protein concentrations and cell counting in bronchoalveolar lavage fluid

In order to investigate the molecular mechanisms underlying acute lung injury after smoke inhalation, we established the ALI rat model, which was induced by smoke inhalation. Compared with the control group, the 6- and 24-h groups subjected to smoke inhalation for 15 min exhibited significant severe pulmonary edema in lung tissues, and an even more severe edema was observed in the 24-h group in comparison to the 6-h group, showing the induction of lung injury by smoke inhalation. To directly show the extent of the injury, the bronchoalveolar lavage fluid (BALF) was collected from all three groups. Our results showed that the total protein concentrations of the 6- and 24-h groups were remarkably higher than those of the control group (Fig. 1a), showing the increased pulmonary vascular permeability caused by smoke inhalation. In addition, the numbers of macrophages (MAC), neutrophils (NEU), and red blood cells (RBC) in the two groups subjected to smoke inhalation were greatly elevated compared with the control group, and were much higher in the 24-h group than the 6-h group (Fig. 1b–d). These results indicate that smoke inhalation induced significant acute lung injury in our rat models.

Smoke inhalation induces severe injury in rat lung tissues

The lung tissue of rats from the three groups was collected, sliced, and stained with hematoxylin-eosin (HE) to ascertain the smoke-induced injury. The stained slices showed that smoke inhalation caused significant disruption of the cell lining in the 6- and 24-h groups, indicating lung tissue injury induced by smoke inhalation (Fig. 2). The lung wet/dry weight ratios (W/D ratio) were then measured to evaluate the pulmonary vascular permeability, and the results showed that the W/D ratios in rats exposed to smoke inhalation were markedly higher than those of the control group (Fig. 3a). Furthermore, the levels of three inflammatory factors,

Table 1 The primers used in this study

| Name	Primers (5′-3′)	Product length (bp)
chr1:164931730|164947123-C	F: AGATGATGCAGACCCATCGC R: CTCAGCTTTCTCAGGCATCCA	149
chr1:164931730|164947123-L	F: TGCACGTCTCTCGGATTCAG R: TGGAAACAAAGGCTGAGCGA	80
chr19:49158646|49159228-C	F: AGGAGACCAGTGATTGCGAC R: GCCGTAGCCTTTCCATCGG	129
chr19:49158646|49159228-L	F: GGGTCTTCAGGAAAGGCTCC R: GTCTCCTGCTCTCCAAGCTG	206
chr6:72537858|72538290-C	F: TCACAGGAGAGCTGCACAAT R: GTCTGTCCGCTTGCGTTCTT	120
chr6:72537858|72538290-L	F: AGACCAACTCGGGAAAGCAG R: TCAGGACTTGAGAACGCACC	80
chr9:73798311|73799430-C	F: CCACCGATAGCAGCTCAGAT R: ACCCTTTGTTGTTGCCTCCT	126
chr9:73798311|73799430-L	F: TACCTCCCCTCAGGCTTCAA R: CTGAACGCTGCTGCCATTAC	157
chr1:78369712|78370333-C	F: AGTGAGAAGGGTCACCGCA R: CGACTCCTCTCTTGTCCTCCA	138
chr1:78369712|78370333-L	F: CACAAACGCCATGCTTCCTC R: CCTTCTCACTGGGGCTGAAA	76
chr11:34612627|34614139-C	F: TTCTCGTGCTGCTGAGATAAGT R: TCATCTGCCAAACTGAGACCT	137
chr11:34612627|34614139-L	F: GAGGGAGGTCTCAGTTTGGC R: TTGCATACCCACGCCTTCAT	138
chr2:224802843|224803455-C	F: GCTATAACAGTCTCAGCACCGA R: GTGTTGGCCTCCATCGTCCT	166
chr2:224802843|224803455-L	F: TCTGCTGCAGCTTTGTCACT R: TCTTCTCGGTGCTGAGACTG	102
chr3:94396940|94398038-C	F: ACTTTGGATCGGCCAGTCAT R: CCGCTGGGCTCTACTTTCAG	164
chr3:94396940|94398038-L	F: GAAAGTAGAGCCCAGCGGTT R: GCCTGAGCTGCTATGACCTT	202
chr11:79763901|79778947-C	F: GAACACAGGCTCAAGGACAGA R: AGGGTGTGGCCTACTTTCCA	161
chr11:79763901|79778947-L	F: TAGAGTGTCTGAAGAGGCTGG R: GAGCTGAGGCACTCTCTCTTC	111
chr1:251455609|251463748-C	F: ATTGCATCGGGGCAAGTTTT R: GGCGGTGTCATAATGTCTCTCA	149
chr1:251455609|251463748-L	F: AAAGCTGGGAAAGGACGGAC R: CAGGGCCTCTTGTGCCTTTA	84

C circular RNA, *L* linear RNA
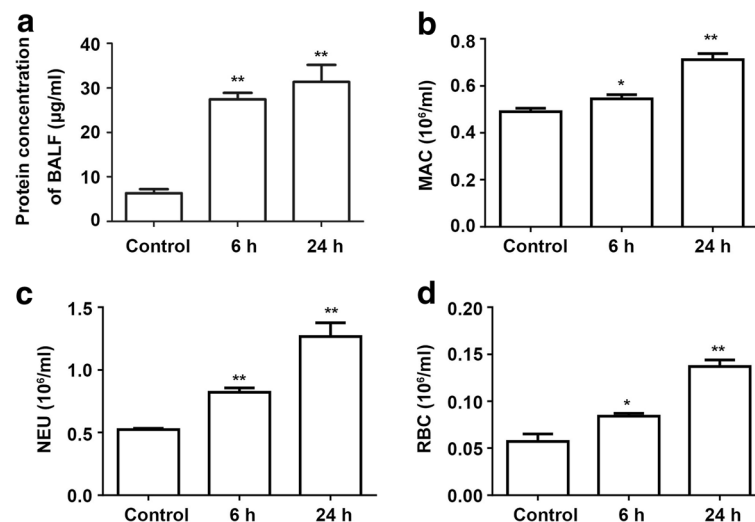
Fig. 1 Protein concentration and cells in bronchoalveolar lavage fluid. **a** Protein concentration in bronchoalveolar lavage fluid of rats after smoke inhalation. The BALF protein concentrations in the 6- and 24-h groups were determined by the BCA method. **b, c** Cell numbers in the broncho-alveolar lavage fluid of rats after smoke inhalation. The numbers of macrophages (**b**), neutrophils (**c**), and red blood cells (**d**) in the BALF from the control and 6- and 24-h groups. BALF: bronchoalveolar lavage fluid; MAC: macrophage; NEU: neutrophil; RBC: red blood cell; *P < 0.05 vs. control, **P < 0.01 vs. control

Fig. 2 Rat lung tissue injury caused by smoke inhalation. Damage to rat lung tissues induced by smoke inhalation. The slices of rat lung tissues were stained with hematoxylin-eosin (HE) and photographed under microscopy. Scale bar 100 μm
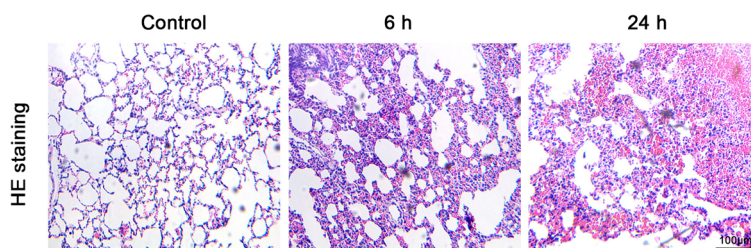
interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and IL-8, in the lung tissues of rats after smoke inhalation were significantly higher than those of the control group (Fig. 3b–d). These dramatic changes in the histology and molecular indexes further confirmed the significant acute lung injury caused by short-term smoke inhalation.

Differentially expressed circRNAs in rat lungs induced by smoke inhalation

To explore the possible involvement of circRNAs in acute lung injury, the differentially expressed circRNAs between the rat lung tissues of the 24-h group and the control group were identified. As shown in Table 2, a total of ten circRNAs were found to be differentially expressed between the rats with smoke inhalation and the control group by next-generation sequencing. This included five significantly upregulated and another five significantly downregulated circRNAs after smoke inhalation, compared with the control group. These differentially expressed circRNAs were found to be associated in multiple pathways including endocytosis, the Rap1 signaling pathway, cancer-related pathways, focal adhesion, and the phosphatidylinositol and sphingolipid signaling pathways. For further verification of the existence and differential

expression of these circular RNA molecules in rat lungs, quantitative PCR (Q-PCR) was carried out. These results showed four upregulated (Fig. 4a) and five downregulated circRNAs (Fig. 4b), confirming nine out of the ten differentially expressed circRNAs that were identified by sequencing, which was highly consistent with the sequencing results. These findings confirmed the prevalent distribution of circRNAs in rat lung tissue during acute lung injury, suggesting that the regulation of gene expression mediated by the change in specific circRNA expression might play an important role in the pathological process of lung injury after smoke inhalation.

Validation of the circRNAs in rat lung tissue

In order to further validate whether the identified circRNAs were indeed circular, RT-PCR methods using different combinations of primers specific for the amplification of either linear or circular RNA molecules were performed. The genomic DNA and the GADPH gene were applied as the controls. The RT-PCR results showed that all four of the tested circRNAs in the assay existed in the form of circular loops in the lung tissue of rats exposed to smoke inhalation (Fig. 5a, b). The ligation sites of these four circRNAs were also
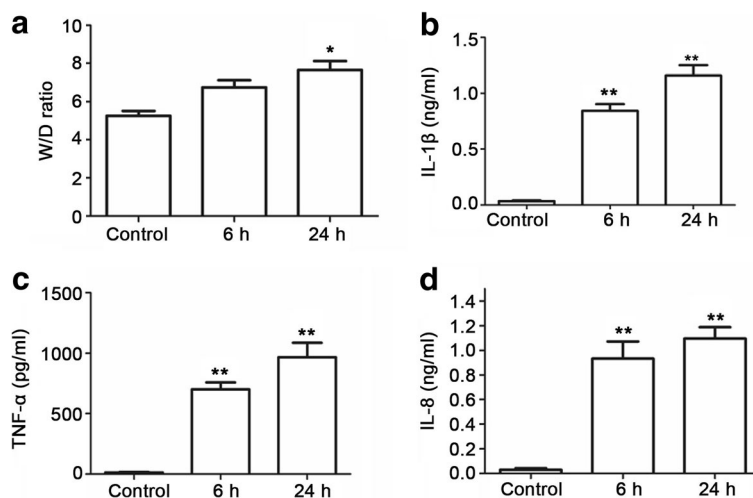
Fig. 3 **a** Lung wet/dry weight ratios (W/D ratios) of rats after smoke inhalation. The wet lung tissues were weighed and then dried at 60 °C for 48 h, whereupon they were again weighed and the ratio was calculated. **b–d** Levels of three inflammatory factors in rat lung tissues after smoke inhalation. The contents of IL-1β (**b**), TNF-α (**c**), and IL-8 (**d**) in rat lung tissues were measured by enzyme-linked immunosorbent assay. HE: hematoxylin-eosin; W/D ratio: wet weight/ dry weight ratio; IL-1β: interleukin-1beta; TNF-α: tumor necrosis factor-alpha; IL-8: interleukin-1beta; *$P < 0.05$ vs. control, **$P < 0.01$ vs. control

**Table 2** Significantly differential circRNAs among control rats and the 24-h ALI rats

| circRNA	Gene ID	Regulation	P value
chr1:164931730|164947123	Rnf169	Down	7.09E−164
chr1:167098354|167098455	Rnf121	Down	1.0229E−98
chr19:49158646|49159228	Cdyl2	Down	1.2007E−89
chr6:72537858|72538290	Hectd1	Down	3.7253E−88
chr11:79763901|79778947	U7,SNORA17	Down	6.9074E−80
chr11:34612627|34614139	Ttc3	Up	1.397E−110
chr2:224802843|224803455	SNORA24,U6atac	Up	2.1908E−86
chr3:94396940|94398038	Hipk3	Up	1.2832E−87
chr9:73798311|73799430	Kansl1l	Up	1.2789E−73
chr1:251455609|251463748	Pten	Up	5.92E−46

circRNA circular RNA, *Down* downregulated, *Up* upregulated

validated by sequencing the RT-PCR products (Fig. 6). Since circRNAs are resistant to RNase digestion, the expression levels of these four circRNA molecules were measured by quantitative PCR after RNase digestion. The linear RNAs
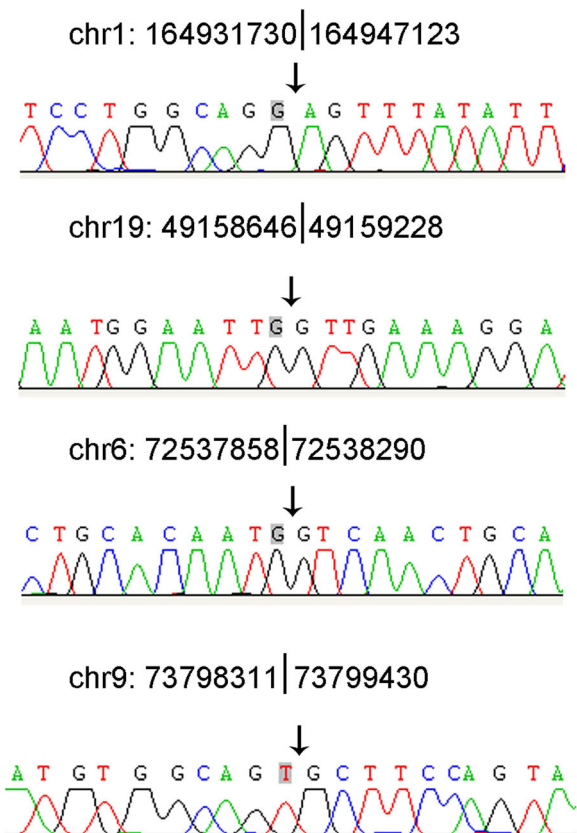

Fig. 4 Differentially expressed circRNAs in rat lung tissue after smoke inhalation. **a** Upregulated circRNAs in rat lung tissues associated with smoke inhalation. The expressions of five upregulated circRNAs were tested by Q-PCR. **b** Downregulated circRNAs in rat lung tissues associated with smoke inhalation. The relative expressions of five downregulated circRNAs were determined by Q-PCR. *$P < 0.05$ vs. control, **$P < 0.01$ vs. control
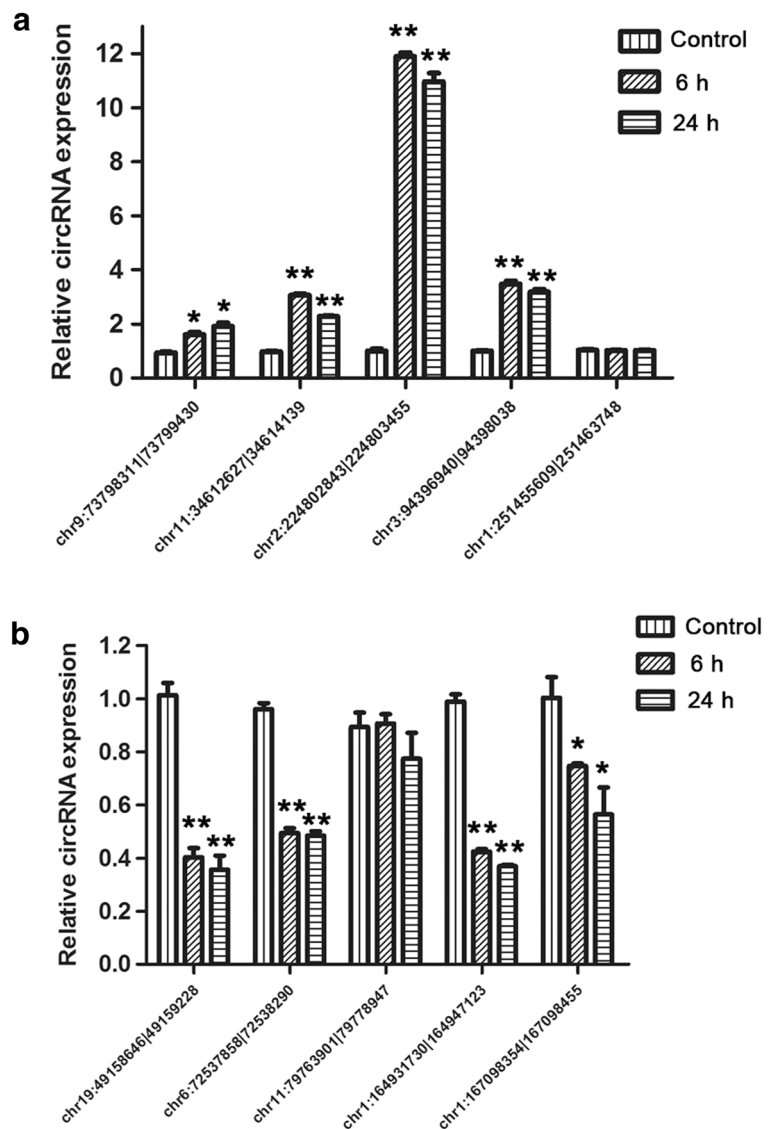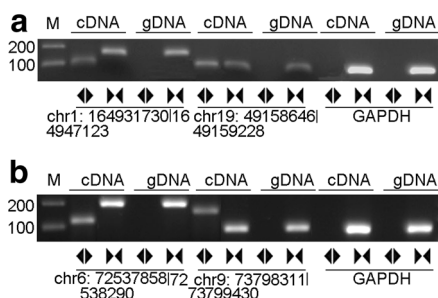
Fig. 5 Validation of the circular form of differential circRNAs. RT-PCR analysis of circRNAs (**a** chr1: 164931730‖164947123, chr19: 49158646‖49159228; **b** chr6: 72537858‖72538290 and chr9: 73798311‖73799430) using different primer combinations. The circular properties of the circRNAs were tested using RT-PCR with primers specific for circular molecules

showed a significant decrease in expression induced by the RNase treatment and were used as the controls. The quantitative PCR combined with RNase digestion also supported the conclusion that these four circRNA molecules identified by our next-generation sequencing assay were truly in the circular loop form (Fig. 7a–d). These results also demonstrate that the method of circRNA identification used in this study provided reliable results, further supporting the prevalence of


Fig. 6 The ligation sites of the circRNAs in rat lung tissues. The ligation sites of four differentially expressed circRNAs in rat lung tissues were analyzed with next-generation sequencing

circRNAs in rat lung tissues during the progression of acute lung injury associated with smoke inhalation.

Discussion

Acute lung injury (ALI) is a severe clinical syndrome associated with high morbidity and mortality, but the underlying mechanisms remain unclear, which limits the development of effective prevention and treatment strategies, in spite of decades of intense investigation [9]. The investigation of the mechanism of lung injury has been greatly challenged due to a variety of clinical variables that could not be properly controlled for during clinical tests in severely affected human patients. The study of acute lung injury using animal models, as a link bridging the gap between the laboratory bench and patients, has been accepted as a feasible and promising strategy for both the research of basic mechanisms and validation of clinical treatments [12]. A number of animal species, including mice and rats, have been applied to investigate lung injury, but factors such as the research focus and animal size should be carefully considered during the choice of proper animal species, due to the species differences like innate immune response [12]. The pulmonary edema in rat models with acute lung injury was revealed to be mainly caused by the increased permeability of post-alveolar venules [10], which is similar to human acute lung injury associated with smoke inhalation. Therefore, we chose rats as the model for our study, with consideration of the proper size required for the surgical operation and sufficient tissue volume for the lung RNA sample extraction. In this study, the subsequent analysis of the bronchoalveolar lavage fluid and lung tissues indicated the successful establishment of acute lung injury symptoms in rats that underwent smoke inhalation, and were applied for the identification and characterization of circRNAs.

The acute response of lung tissues to damage is controlled by a network of complex molecular pathways, which is not well understood. Previous extensive research has shown that microRNA (miRNA) has emerged as a novel class of gene expression regulators during the progression of acute lung injury [21, 24]. For instance, the treatment of antisense oligonucleotides (ASOs) against miR-155 was shown to promote the recovery of acute lung injury in mice, mediated by IL-10-secreting M2-like macrophages [7]. Another microRNA molecule, miR-17, was found to regulate the overexpression of FoxA1 during acute lung injury in the murine model induced by lipopolysaccharide (LPS) treatment, and the increased FoxA1 expression after ALI by miR-17 might play a critical role in ALI progression through promoting alveolar type II epithelial cell apoptosis [21]. The association between miRNAs and the related target genes with acute lung injury has been supported by a large number of
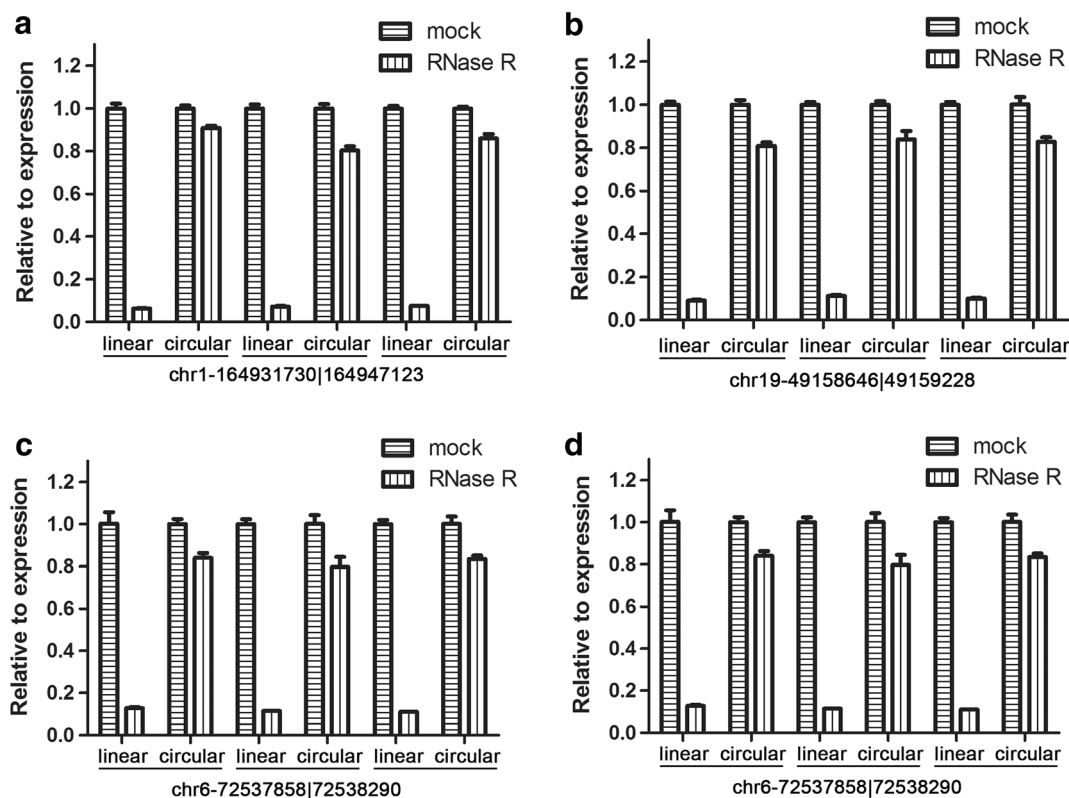
Fig. 7 The resistance of circular RNAs to RNase digestion. The expressions of chr1: 164931730‖164947123 (**a**), chr19: 49158646‖49159228 (**b**), chr6: 72537858‖72538290 (**c**) and chr9: 73798311‖73799430 (**d**) were tested by Q-PCR following RNase digestion. The corresponding linear RNA and GAPDH were used as the controls. GAPDH: glyceraldehyde-3-phosphate dehydrogenase

studies using both animal and cellular model systems, which were recently well summarized [17]. As discussed above, circRNAs have recently been identified as a novel group of non-coding RNA molecules regulating gene expression as miRNA sponges [8], indicating that they might also be involved in the process of acute lung injury associated with smoke inhalation, considering the close linkage between miRNA and ALI.

However, the expression of circRNA molecules and their potential regulatory role in acute lung injury have never been addressed. In the present study, we comprehensively identified the differentially expressed circular RNAs in rat lung tissues after smoke inhalation-induced acute lung injury. The significant differences in the expressions of these circRNAs between the ALI and the control groups suggested that these circRNAs might play important regulatory roles during smoke inhalation-induced acute injury in rat lung tissues. Furthermore, the circular shape of these molecules was validated by multiple assays based on RT-PCR methods. These findings provide an important research basis for the future investigation of the pathological and molecular mechanisms underlying the regulation of the progression of acute lung injury by these specific circRNA molecules. It should also be noted that this study is limited by the lack of further

investigation into the functions of the identified differentially expressed circRNAs, as well as the underlying molecular mechanisms, due to limited research grants. Future studies of the genes regulated by these circRNAs might bring novel insights into the signaling pathways responsible for the progression of acute lung injury associated with smoke inhalation. In addition, the discoveries in this study might also be applied to the exploration of other lung diseases with a similar pathological process as ALI.

To address the possible involvement of circRNA molecules in the progression of acute lung injury caused by smoke inhalation, we constructed the rat model of ALI by treatment with smoke inhalation, which was validated by the dramatic change of bronchoalveolar lavage fluid and lung tissues. Through next-generation sequencing, a number of differentially expressed circRNAs were identified from the lung tissues of rats with acute lung injury caused by smoke inhalation. The circular form of these identified novel circRNA molecules was confirmed by multiple RT-PCR-based assays. The new discovery of the existence of differential circRNAs in the progression of acute lung injuries suggests a potential key role of circRNAs in ALI associated with smoke inhalation, which might provide clues for the diagnosis and treatment of acute lung injury associated with smoke inhalation.

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