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Analysis of Differentially Expressed MicroRNAs in OVA-induced Airway Remodeling Mice Model

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ABSTRACT

MicroRNAs (miRNAs) can participate in airway remodeling by regulating immune molecule expression. Here, we aimed to identify the differential miRNAs involved in airway remodeling.

Airway remodeling was induced by ovalbumin in female BALB/C mice. The differentially expressed miRNAs were screened with microarray. GO (Gene Ontology) and KEGG enrichment analysis was performed. The miRNA target gene network and miRNA target pathway network were constructed. Verification with real-time PCR and Western blot was performed.

We identified 63 differentially expressed miRNAs (50 up-regulated and 13 down-regulated) in the lungs of ovalbumin-induced airway remodeling mice. Real-time PCR confirmed that 3 miRNAs (mmu-miR-1931, mmu-miR-712-5p, and mmu-miR-770-5p) were significantly up-regulated, and 4 miRNAs (mmu-miR-128-3p, mmu-miR-182-5p, mmu-miR-130b-3p, and mmu-miR-20b-5p) were significantly down-regulated. The miRNA target gene network analysis identified key mRNAs in the airway remodeling, such as *Tnrc6b* (trinucleotide repeat containing adaptor 6B), *Sesn3* (sestrin 3), *Baz2a* (bromodomain adjacent to zinc finger domain 2a), and *Cux1* (cut like homeobox 1). The miRNA target pathway network showed that the signal pathways such as MAPK (mitogen-activated protein kinase), PI3K/Akt (phosphoinositide 3-Kinase/protein kinase B), p53 (protein 53), and mTOR (mammalian target of rapamycin) were closely related to airway remodeling in asthma.

Collectively, differential miRNAs involved in airway remodeling (such as mmu-miR-1931, mmu-miR-712-5p, mmu-miR-770-5p, mmu-miR-128-3p mmu-miR-182-5p, and mmu-miR-130b-3p) as well as their target genes (such as *Tnrc6b*, *Sesn3*, *Baz2a*, and *Cux1*) and pathways (such as MAPK, PI3K/Akt, p53, mTOR pathways) have been identified. Our findings may help to further understand the pathogenesis of airway remodeling.

Keywords: Airway remodeling; Computational biology; MicroRNAs; Microarray analysis

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INTRODUCTION

Asthma is a common chronic respiratory disease. Many studies have shown that airway remodeling is an important pathological process of asthma and is independent of inflammation.[1,2] Most studies only confirmed the known biomarkers of asthma but did not identify any new diagnostic markers or treatment targets. Therefore, we should study the pathogenesis of airway remodeling at the gene level rather than just focus on the immune response.

The identification of naturally occurring noncoding RNA molecules, such as microRNAs (miRNAs), has made it possible to understand and treat diseases.[3] Such small RNA molecules can directly inhibit or induce target mRNA degradation and have a major function in the fine-tuning of gene expression.[4] MiRNAs can regulate the oxidative stress response by changing their ability to bind to the target mRNA sequences. In addition, miRNAs are also involved in various important biological processes, such as immune response, cell differentiation, development, and apoptosis.[5,6] For instance, the abnormal expression of miRNAs is related to the occurrence of lung diseases (e.g., asthma) in children and adults.[7] The miR-142-5p and miR-130a-3p can regulate lung macrophage polarization and asthmatic airway remodeling.[8] MiR-133a can reduce asthmatic airway remodeling through the PI3K/AKT/mTOR signaling pathway.[9,10] Scholars have sequenced miRNA profiles of CD4+ T cells in the spleen of a mouse model of acute asthma.[11] However, the miRNA profile of the lung tissue of ovalbumin (OVA)-induced asthmatic mice has not been reported.

Gene chip is a technology in which a large number of full-length or fragments of target genes are fixed and arranged on a glass slide, silicon wafer, or nylon membrane solid support in an orderly and high-density manner.[12] It has the characteristics of high throughput, multiple parameters, and parallelization. There are few reports on the use of gene chips to screen the miRNA profile in mice with OVA-induced asthma. Here, we screened and identified the differential miRNAs involved in asthma. Gene chip technology was used to detect the difference in miRNAs between normal mouse lung tissue and the lung tissue of mice with OVA-induced airway remodeling. Preliminary screening of these differentially expressed miRNAs may help to further study the occurrence and development of airway remodeling and provide possible targets for the treatment of asthma.

MATERIALS AND METHODS

Animals

The female BALB/C mice (6-8 weeks old; n=6; body weight, 18±2 g) were purchased from the Experimental Animal Center of Yanbian University. They were housed in standard conditions. All animal procedures were approved by the Ethics Committee of Yanbian University (Approval number: JN. No. 20200612b0681230).

Establishment of Asthma Model

Mice were randomly divided into 2 groups, with 3 mice in each group. Mice in the model group were given an intraperitoneal injection of 0.5 mL OVA (Sigma, USA, 2 mg/mL) on days 1, 7, and 14 for sensitization. The mice in the control group were treated with the same amount of PBS. From day 17, mice in the model group were given aerosol inhalation of 1% OVA for excitation 3 times a week, 30 min each time. Mice in the Control group were challenged with the same amount of PBS. After anesthesia, the mice were sacrificed, and the lung tissues were collected.

Histological Staining

The left lung tissue was fixed in 4% formaldehyde for 24 h, embedded, and cut into sections. Hematoxylin-eosin (H&E) staining was performed to analyze bronchus morphology. Periodic acid Schiff (PAS) staining assessed goblet cell proliferation and mucus production. Masson Trichrome staining evaluated the degree of fibrosis in lung tissue. All staining was performed according to routine procedures.

Quantitative Real-time PCR

The RNA samples were obtained from lung tissues with an RNA isolation kit (TIANGEN, DP451, Beijing, China), and then reverse transcription was performed with a reverse transcription kit (TIANGEN, KR118-02). The quantitative real-time PCR was performed with SYBR green quantitative real-time PCR reagent (TIANGEN, KR123) on CFX96 Touch™ Real-Time PCR Detection System (BIORAD). The bulge-loop RT primer and qPCR primers specific for mmu-miR-1931, mmu-miR-712-5p, mmu-miR-770-5p, mmu-miR-128-3p, mmu-miR-182-5p, mmu-miR-130b-3p, and mmu-

miR-20b-5p were designed and synthesized by Ribo Bio (Guangzhou, China). The U6 gene was used as an internal control. The expression level was calculated by the $2^{-\triangle\triangle Ct}$ method.

Western Blot

Total protein was extracted from mouse lungs after homogenization with a fully automatic sample crusher (TissuelyserII, Qiagen, Germany) and lysis with RIPA buffer and protease inhibitor K (Beyotime, Jiangsu, China). The protein (10 μg each lane) was separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membrane was probed with primary and secondary antibodies. The primary antibodies were phosphorylated p38 mitogen-activated protein kinase (p-p38MAPK, #4511, Cell Signaling Technology, Germany), p38MAPK (#8690, Cell Signaling Technology, Germany), phosphatidyl-inositol-3,4,5-trisphosphate (p-PI3K, #4228, Cell Signaling Technology, Germany), PI3K (#4249, Cell Signaling Technology, Germany), phosphorylated protein kinase B (p-Akt, #4060, Cell Signaling Technology, Germany), Akt (#4691, Cell Signaling Technology, Germany), protein 53 (p53, #2524, Cell Signaling Technology, Germany), and mammalian target of rapamycin (mTOR, #2972, Cell Signaling Technology, Germany). The secondary antibody was goat antirabbit IgG antibody-conjugated horseradish peroxidase (#7074, Cell Signaling Technology, Germany). After color development with ECL Western Blotting Substrate (Thermo, 32109, USA), the blots were scanned on a gel imager (Amersham Imager 600, USA).

Microarray

Total RNA was extracted from lung tissue samples using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) and purified with the RNeasy mini kit (Qiagen, Valencia, CA, USA). Biotinylated cDNA was prepared according to the standard Affymetrix protocol from 250 ng total RNA using Ambion WT Expression Kit (Thermo Fisher Scientific, USA). Following labeling, fragmented cDNA was hybridized for 16 h at 45 °C on GeneChip miRNA 4.0 Array (Affymetrix). GeneChips were washed and stained in the Affymetrix Fluidics Station 450 (Thermo Fisher Scientific, USA). All arrays were scanned using Affymetrix GeneChip Command Console (AGCC) installed in GeneChip Scanner 3000 (7G). The differentially expressed miRNAs were screened according to the criteria of $p<0.05$ and fold change>1.5.

Gene Ontology (GO) Enrichment Analysis

GO analysis was performed using Gene ontology (http://geneontology.org/). Fisher's exact test and Benjamini-Hochberg were used, and the false discovery rate was calculated to correct the *P*-value. The difference screening criterion was $p<0.01$.

KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis was performed with online software (http://www.kegg.jp/). Fisher's exact test and chi-square test were used to identify the significance pathway. False discovery rates *p* values were used to determine the significance threshold. The difference screening criterion was $p<0.05$.

Construction of miRNA Target Pathway Network

The miRNA target pathway network was constructed according to the relationships between significant pathways and genes, as well as the relationships among miRNAs and pathways. The visualization of the network was performed by Cytoscape (vension:3.6.0).

Construction of miRNA Target Gene Network

We used the relationship between miRNA and its target Gene attribute to establish the miRNA-gene action network. The network was visualized by Cytoscape (vension:3.6.0).

Statistical Analysis

Data are represented as mean ± SD of at least three experiments. Significant differences were assessed by Student's t-test and $p<0.05$ was considered statistically significant.

RESULTS

Pathological Changes in Lung Tissue in OVA-induced Airway Remodeling Mice

As shown in Figure 1A, we found that in H&E staining, the bronchial structure of the lung tissue of the control group was clear and the airway walls were thin, uniform, and intact. However, the lung tissue of mice with OVA-induced airway remodeling showed

extensive peribronchial and perivascular inflammatory cell infiltration. Furthermore, the structure of the bronchial and alveolar walls was severely damaged. Then, we performed PAS staining on mouse lung tissue to observe the proliferation of goblet cells. The results showed that the epithelial goblet cells of the mice in the model group were positive for PAS staining, which was more than that in the control group (Figure 1B). Finally, we used Masson Trichrome staining to evaluate the degree of collagen deposition and fibrosis in the lung tissue. In the Control group, there were no obvious pathological changes in the lung tissue and no fibrous tissue hyperplasia. However, the airway and interstitium of the model group had obvious collagen deposition and fibrosis (Figure 1C). The above results suggest that the mice models of asthma airway remodeling were successfully established, as indicated by inflammatory cell infiltration, goblet cell hyperplasia, and increased collagen deposition and fibrosis.

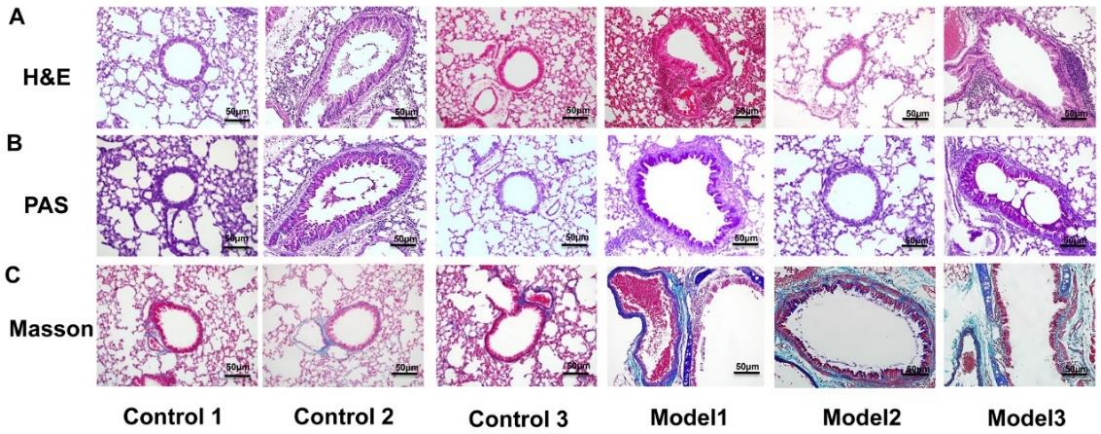

Figure 1. Pathological changes in lung tissue. Hematoxylin-Eosin (H&E) staining (A), Periodic Acid-Schiff (PAS) staining (B), and Masson Trichrome staining (C) were used to detect whether the model was successfully constructed. Representative images from 3 mice of each group were shown. 100× magnification.

Differential Expression of miRNAs in OVA-induced Airway Remodeling Mice

We analyzed the expression of miRNAs with microarray and used the Relevance Vector Machine RVM t-test to screen differentially expressed miRNAs. As shown in Figure 2A, the model group had 50 up-regulated miRNAs and 13 down-regulated miRNAs compared with the control group. Figure 2B shows the unsupervised hierarchical cluster analysis of the two data sets. In the OVA-induced airway remodeling model, mmu-miR-714, mmu-miR-1949, mmu-miR-1898, mmu-miR-3081-5p, and mmu-miR-1934-3p were significantly up-regulated compared with the control group (P<0.05), while mmu-miR-15b-5p, mmu-miR-92a-3p, mmu-miR-1195, mmu-miR-128-3p, mmu-miR-182-5p were significantly down-regulated (p<0.05). We selected 7 differential miRNAs closely related to allergic inflammation and asthma for qRT–PCR (Figure 2C). We confirmed that 3 miRNAs (mmu-miR-1931, mmu-miR-712-5p, and mmu-miR-770-5p) were significantly up-regulated and 4 miRNAs (mmu-miR-128-3p, mmu-miR-182-5p, mmu-miR-130b-3p, and mmu-miR-20b-5p) were significantly down-regulated.

GO Analysis of Differentially Expressed miRNAs in OVA-induced Airway Remodeling Mice

GO analysis was used to analyze the main functions of differentially expressed miRNAs. The top 10 enriched terms are shown. For the biological process of GO, the target mRNAs of up-regulated miRNAs were mainly enriched in transcription (DNA-templated), regulation of transcription DNA-templated, regulation of transcription from RNA polymerase II promoter, transport, and negative regulation of transcription from RNA polymerase II promoter (Figure 3A); and, the target mRNAs of down-regulated miRNAs were mainly enriched in nervous system development, transcription (DNA-templated), transport, regulation of transcription (DNA-templated), and positive regulation of transcription from RNA polymerase II promoter (Figure 3B).

For the cellular components of GO, the target mRNAs of up-regulated miRNAs were mainly enriched in the membrane, cytoplasm, nucleus, plasma membrane, and cytosol (Figure 3C). The target mRNAs of down-regulated miRNAs were primarily enriched in the membrane, cytoplasm, plasma membrane, nucleus, and cytosol (Figure 3D).

For the molecular function of GO, the target mRNAs of up-regulated miRNAs were mainly enriched in protein binding, metal ion binding, DNA binding, sequence-specific DNA binding, and transferase activity (Figure 3E). The target mRNAs of down-regulated miRNAs were mainly enriched in protein binding, metal ion binding, DNA binding, transferase activity, and nucleotide binding (Figure 3F).

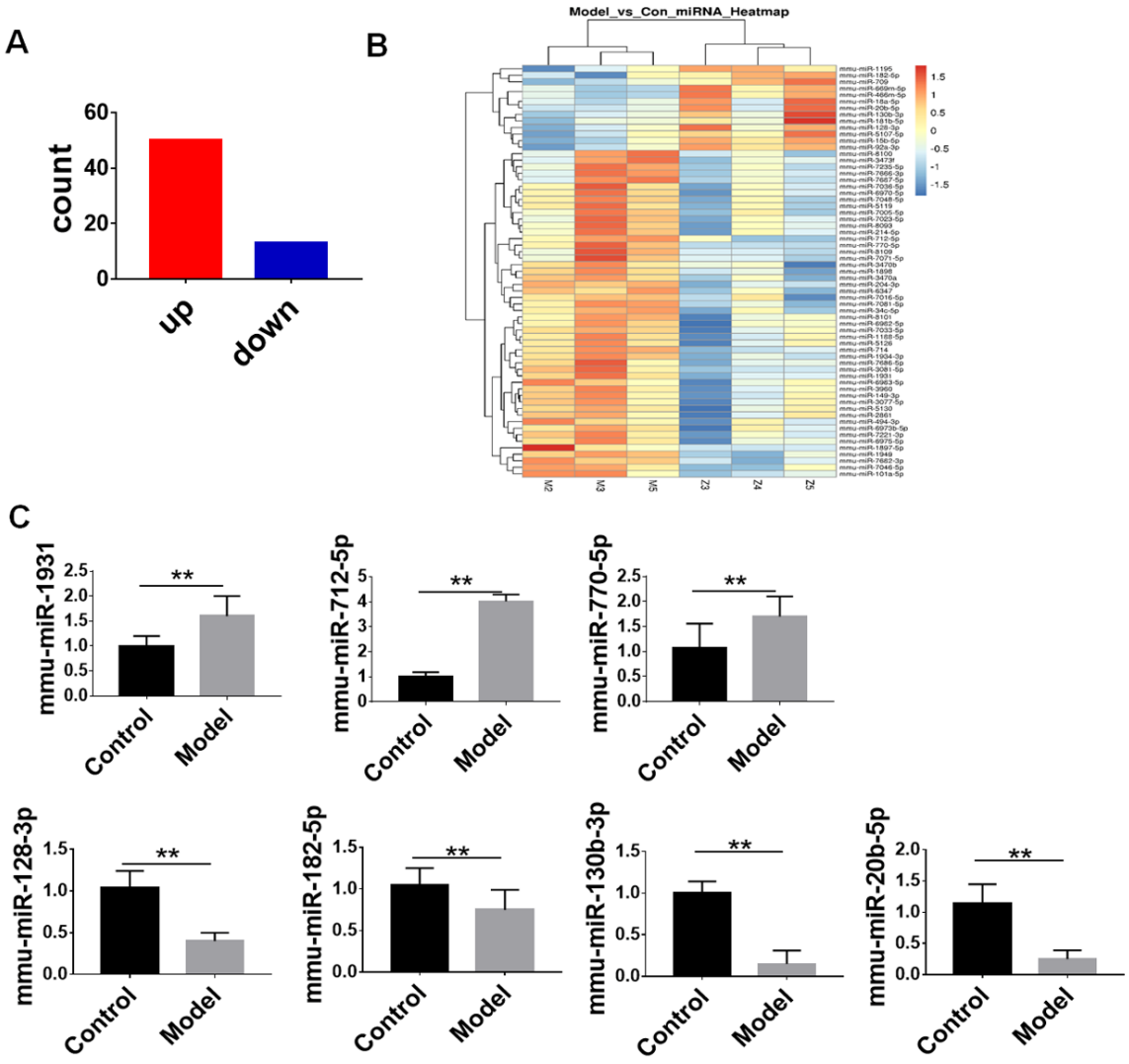

Figure 2. Analysis of differentially expressed MicroRNAs (miRNAs) in lung tissue samples of the control group and airway remodeling group. A) The number of miRNAs differentially expressed between model and control mice (fold change>1.5; *p*<0.05). Red indicates up-regulated miRNAs, and blue indicates down-regulated miRNAs. B) Hierarchical clustering of differentially expressed miRNAs. C) Analysis of differential miRNA with quantitative real-time PCR (*p*<0.01).**

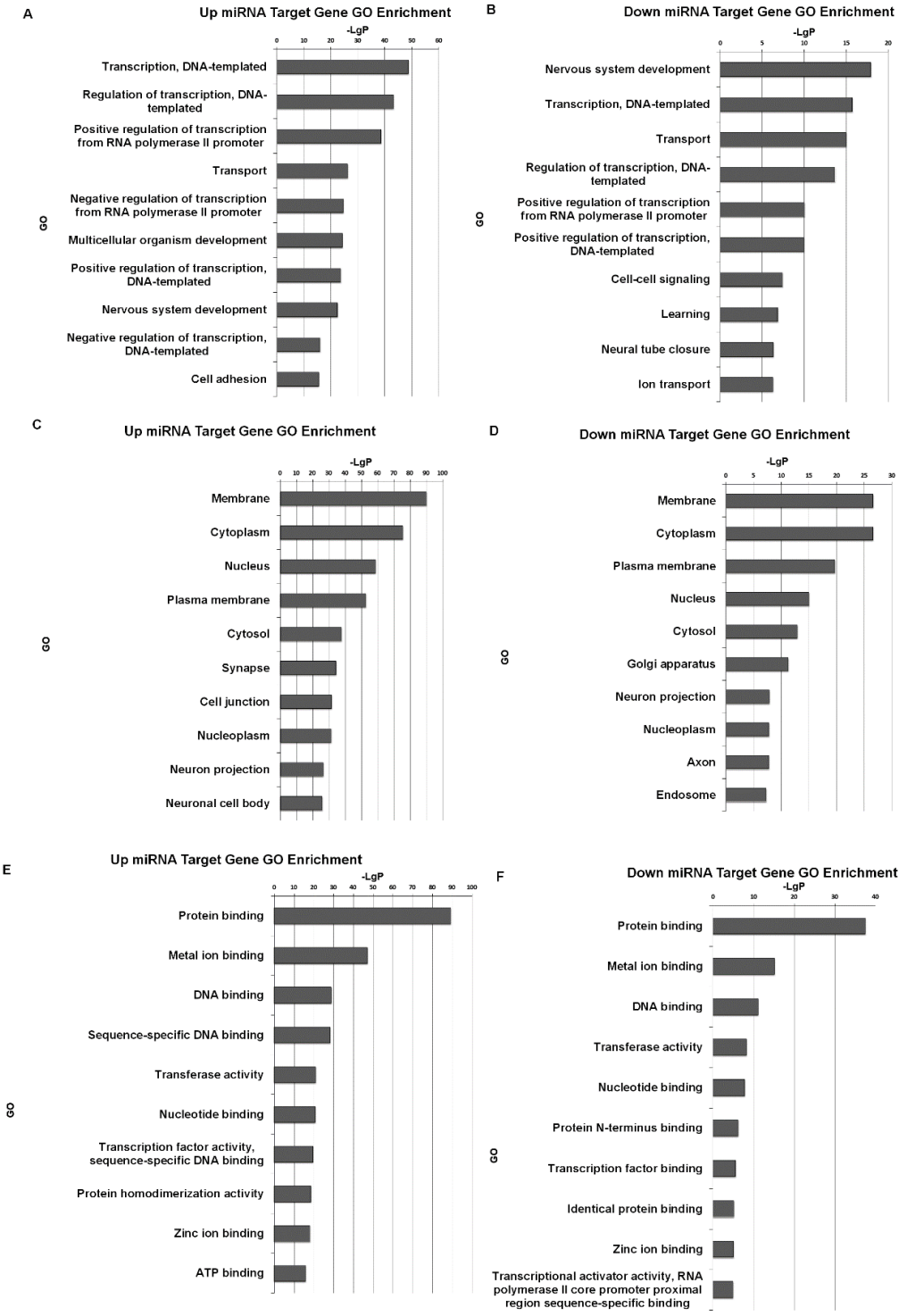

Figure 3. Gene Ontology (GO) analysis of target mRNAs of differentially expressed microRNAs (miRNAs). The top 10 enriched terms are shown. For biological processes, the enriched terms of target mRNAs of up-regulated miRNAs (A) and down-regulated miRNAs (B). For cellular components, the enriched terms of target mRNAs of up-regulated miRNAs (C) and down-regulated miRNAs (D). For molecular function, the enriched terms of target mRNAs of up-regulated miRNAs (E) and down-regulated miRNAs (F). The statistical significance shown on the X-axis is represented with -LgP. A larger -LgP indicates a smaller *p* value. The Y-axis represents the enriched GO terms. The difference screening criterion was $p<0.01$.

KEGG Pathway Analysis of Differentially Expressed miRNAs in OVA-induced Airway Remodeling Mice

Based on the KEGG database, the pathways of differentially expressed miRNAs were analyzed. The top 10 enriched pathways were shown. In Figure 4A, the enriched pathways of the target mRNAs of up-regulated miRNAs included pathways in cancer, Wnt signaling pathway, mTOR signaling pathway, EGFR tyrosine kinase inhibitor resistance, and Th17 cell differentiation. The enriched pathways of the target mRNAs of down-regulated miRNAs included the calcium signaling pathway, adrenergic signaling in cardiomyocytes, pathways in cancer, signaling pathways regulating pluripotency of stem cells, and morphine addiction (Figure 4B).

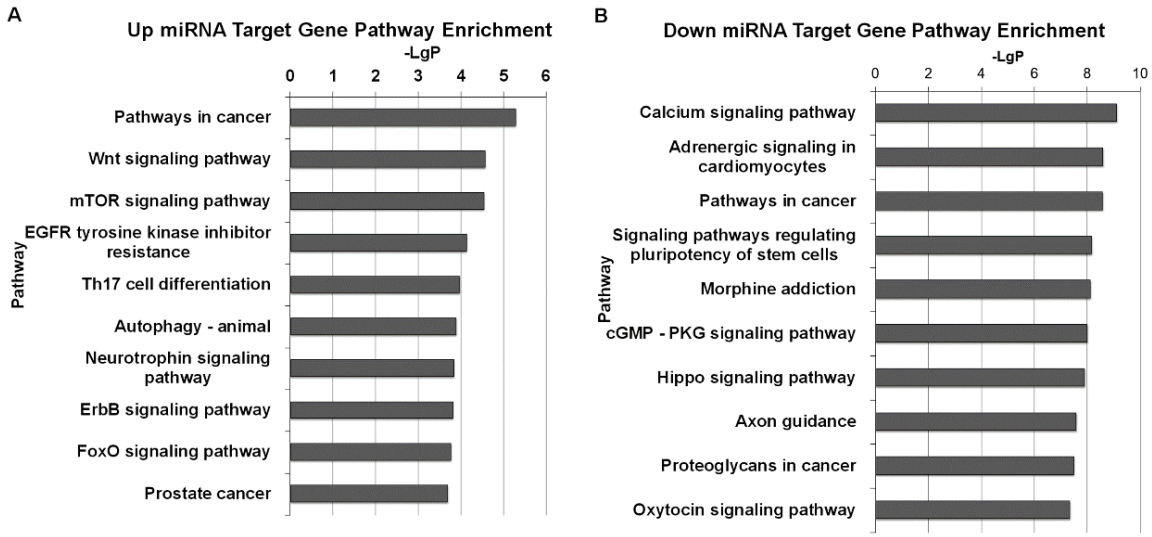

Figure 4. Pathway analysis of target mRNA of differentially expressed microRNA (miRNAs). The Y-axis represents the significantly enriched pathways, and the X-axis represents -LogP (LgP). A larger -LgP indicates a smaller *p* value. (A) The enriched pathways of target mRNAs of up-regulated miRNAs. (B) The enriched pathways of target mRNAs of down-regulated miRNAs. The difference screening criterion was $p<0.05$.

MicroRNA Target Gene Network of Differentially Expressed miRNAs in OVA-induced Airway Remodeling Mice

To screen the core genes for the onset of asthma, we constructed the miRNA target gene network. The size of the dots represents the number of genes. The core genes in the network diagram can be selected for subsequent verification experiments or further analysis. In Figure 5, the larger dots such as *Tnrc6b*, *Sesn3*, *Baz2a*, and *Cux1* were the core genes in the network. Analysis showed that these mRNAs were mainly related to miRNA-mediated inhibition of translation (Tnrc6b), oxidative stress (Sesn3), transcription (Baz2a), and lung development (Cux1). The results show that these miRNAs may be related to molecular functions, such as intercellular signals and interactions, cell growth, proliferation, and gene expression.

MicroRNA Target Pathway Network of Differentially Expressed miRNAs in OVA-induced Airway Remodeling Mice

We constructed a microRNA target pathway network to further understand the key pathways related to these miRNAs. The size of the dots represents the number of pathways. The core pathway in the network diagram can be selected for subsequent verification experiments. The network in Figure 6A reflects the relationship between the target microRNA and the gene pathway. The red boxes represent miRNAs, and the blue dots represent pathways. The main pathways included the MAPK signaling pathway, PI3K-Akt signaling pathway, p53 signaling pathway, and mTOR signaling pathway.

We used Western blot to verify the expression of key proteins in the MAPK signaling pathway, PI3K-Akt signaling pathway, p53 signaling pathway, and mTOR signaling pathway. It was found that the expression of p-p38MAPK, p-PI3K, p-Akt, p53, and mTOR protein increased in the asthma airway remodeling model (Figure 6B), which is consistent with the results of the microRNA target pathway network. The results show that asthma airway remodeling may be closely related to these key pathways.

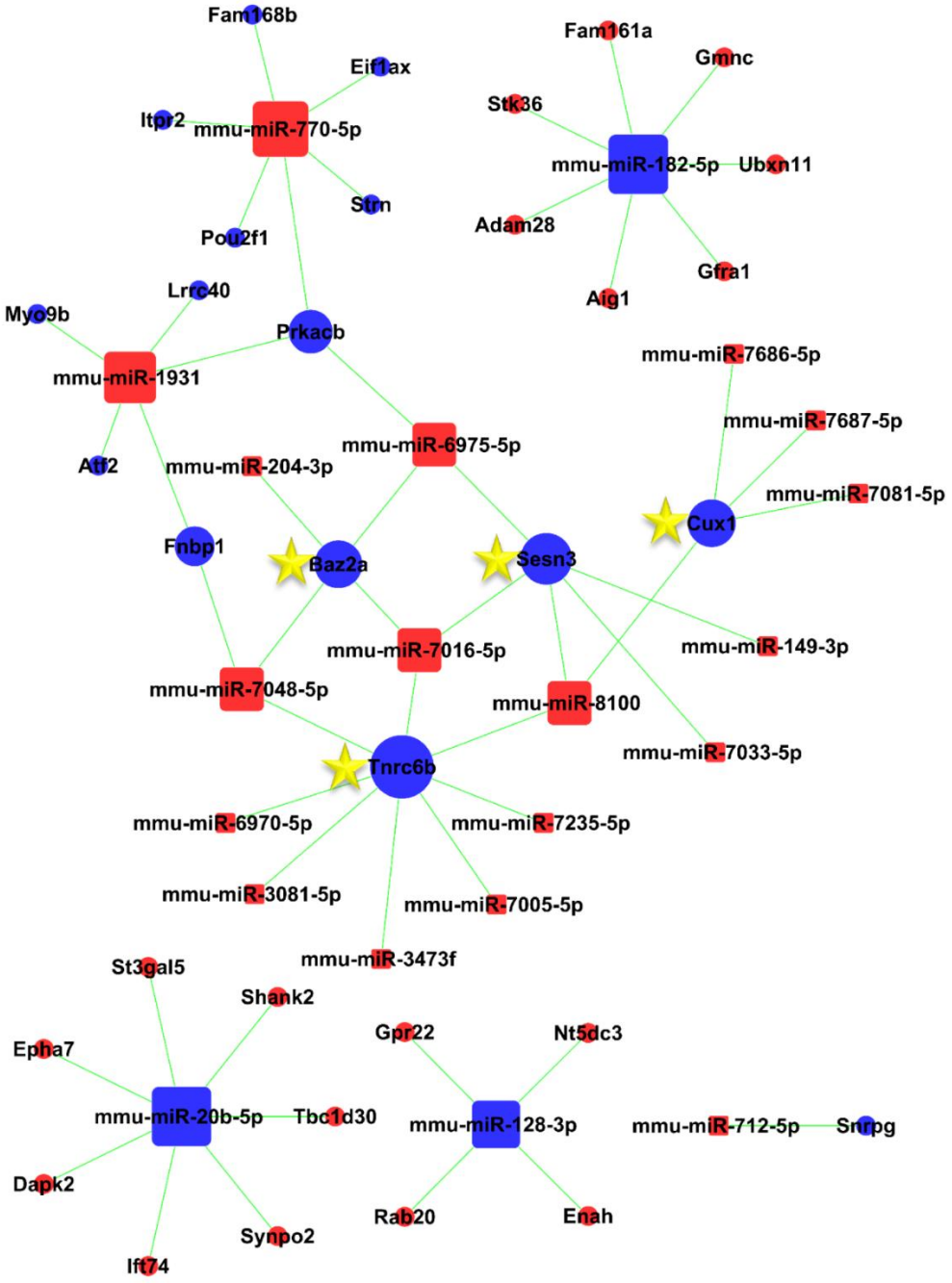

Figure 5. MicroRNA (miRNAs) target gene network. The red box nodes represent miRNAs, and the blue dots represent target genes. The yellow stars represent the mRNAs primarily analyzed in this study. The midpoint size in the network represents the degree of correlation between miRNAs and genes.

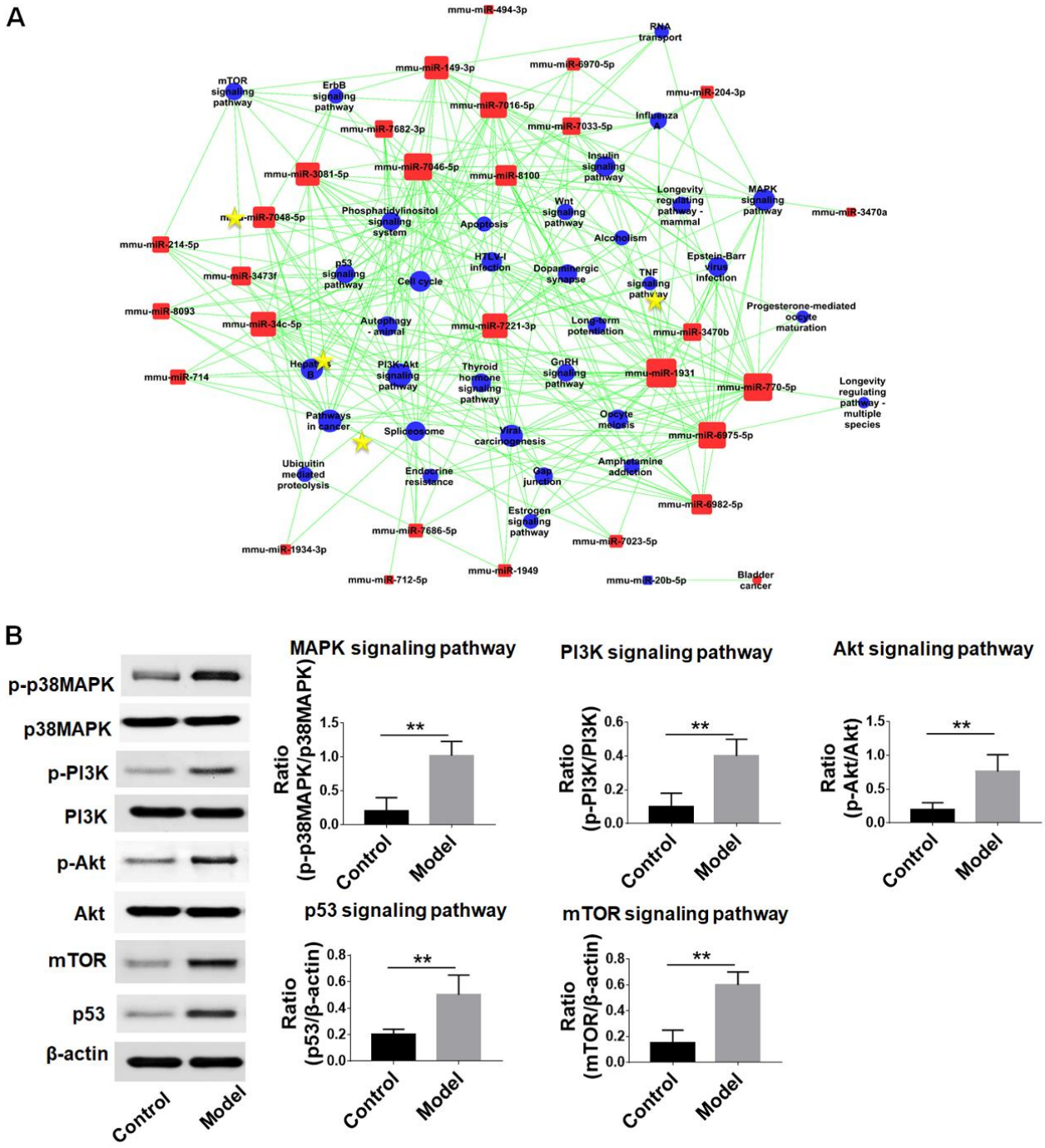

Figure 6. MicroRNA (miRNAs) target pathway network of differentially expressed miRNAs. A) MicroRNA target pathway network. The red box nodes represent miRNAs, and the blue dots represent pathways. The size of the dots represents the correlation between miRNAs and pathways. The yellow stars represent the primarily analyzed pathways in this study. (B) Verification of key proteins in mitogen-activated protein kinase (MAPK) signaling pathway, phosphoinositide 3-Kinase (PI3K)-protein kinase B (Akt) signaling pathway, p53 (protein 53) signaling pathway, and mammalian target of rapamycin (mTOR) signaling pathway (p<0.01).**

DISCUSSION

Bronchial asthma is a heterogeneous airway disease and is easy to recur.[13] Currently, the pathogenesis of asthma includes chronic airway inflammation, airway hyperresponsiveness, and airway remodeling.[14] In this study, we established an airway remodeling model in female BALB/C mice using OVA. H&E staining, PAS staining, and Masson staining showed that the lung tissue of the model mice had obvious airway remodeling, such as airway goblet cell hyperplasia, increased mucus secretion, smooth muscle hyperplasia, basement membrane thickening, epithelial damage, and fibrosis. The above results suggest that the mouse model of asthma airway remodeling has been established successfully.

Although the field of miRNAs has been steadily growing in the past 20 years, the role of miRNAs in the development and regulation of asthma is still relatively unexplored. In this study, the miRNA expression array analysis showed that 63 miRNAs were differentially expressed in the mouse model of asthma airway remodeling. Among them, 50 were up-regulated, and 13 were down-regulated. In addition, we selected 7 of the differential miRNAs for qRT–PCR. These results confirmed that miR-128-3p, miR-182-5p, miR-130b-3p, and miR-20b-5p were down-regulated, whereas miR-1931, miR-712-5p, and miR-770-5p were up-regulated. It has been reported that miR-128-3p can protect mouse lung tissue by inhibiting MAPK14 in the main pathway of asthma.[15] MiR-182-5p is involved in the proliferation and migration of airway smooth muscle cells.[16] MiR-130b-3p participates in epithelial-mesenchymal transition by targeting insulin-like growth factors -1 (IGF-1).[17] Oxidative stress occurs during the onset of asthma, and study has shown that oxidative stress can down-regulate the expression of miR-20b-5p.[18] Macrophage polarization is unbalanced during asthma, and miR-1931 participates in M1 polarization, leading to changes in the immune response and signal transduction.[19] Inflammation is intensified during the inflammatory reaction phase of asthma, and the expression of miR-712-5p is significantly increased in a mouse model of acute lung inflammation.[20] Pulmonary fibrosis is aggravated during airway remodeling, and miR-770-5p inhibits pulmonary fibrosis by targeting TGFBR1.[21] These results verify that miR-128-3p, miR-182-5p, miR-130b-3p, miR-20b-5p, miR-1931, miR-712-5p, and miR-770-5p may play a key role in airway remodeling in asthma.

The role of activation/inhibition of different transcription factors and the genetic regulation of their expression in asthma may be an important aspect of modulating the expression of different clinical phenotypes and their response to treatment.[22] Activation of the neuronal system in allergic airway inflammation has been suggested as a potential immune system modulator in asthma.[23] During the airway remodeling in asthma, changes in various proteins in cells can be found in various components of cells.[24] Insulin growth factor-binding protein-1 may play a novel role in asthma by regulating airway smooth muscle growth.[25] Peroxidase produced by eosinophils can catalyze the breakdown of HOBr, leading to tissue damage associated with inflammatory diseases such as asthma through low-valent transition metal ions.[26] Steroid-resistant asthma patients have a reduced number of receptors available for DNA binding, impairing the ability of glucocorticoid receptors to bind to GRE.[27] Consistently, in this study, GO function enrichment analysis showed that the target mRNAs of differentially expressed miRNAs were involved in biological processes, such as transcription, transport, and nervous system development; in cellular components, such as the cell membrane, cytoplasm, nucleus, plasma membrane and cytoplasm; and in molecular functions, such as protein binding, metal ion binding, DNA binding, sequence-specific DNA binding, transferase activity and nucleotide binding.

Studies have reported the activation of cancer pathways such as JAK-STAT6 and CXCL8-CXCR1/2 in the development of asthma.[28,29] The Wnt signaling pathway is a target for the drug management of severe asthma.[30] Asthma attacks require the activation of the mTOR signaling pathway.[31] EGFR tyrosine kinase inhibitor resistance attenuates IL-6 and IL-8 induced by house dust mite allergen Der p2.[32] Th17 cells can exacerbate the development of asthma.[33] Ca^{2+} regulation mechanisms in airway smooth muscle are a major component of airway hyperresponsiveness.[34] Persistent β2-adrenergic receptor signaling modulates phosphoproteins that alter airway contractility.[35] Human iPSC-MSCs prevent steroid-resistant neutrophilic airway inflammation via modulating Th17 phenotypes.[36] ssCRE-BP/Pur α, a single-stranded DNA-binding protein that may be involved in gene replication, transcription and morphine addiction, is increased when

airway inflammation occurs.[37] In line with these findings, the KEGG pathway analysis of the differentially expressed miRNAs in this study showed that the mainly enriched pathways included Wnt signaling pathway, mTOR signaling pathway, EGFR tyrosine kinase inhibitor resistance, Th17 cell differentiation, calcium signaling pathway, adrenergic signaling in cardiomyocytes, pathways in cancer, signaling pathways regulating pluripotency of stem cells, and morphine addiction.

Through microRNA target gene network analysis, Tnrc6b, Sesn3, Baz2a, and Cux1 were found to be the key protein targets involved in asthma airway remodeling. Tnrc6b is the core protein in the process of RNA interference, and it is a homologous protein with Tnrc6b and Tnrc6c.[38] They are mainly involved in miRNA-mediated translation inhibition, gene silencing by RNA, and other biological functions. Sestrins (Sesn) belong to the family of stress response proteins. Their expressions are up-regulated under stress, and they have a variety of biological functions.[39] Sesn3, a new type of PA26 structurally related gene, is induced by the Fox O family of transcription factors.[40] Baz2a is mainly involved in the transcription process with DNA templates.[41] Cux1 plays an important role in lung development.[42] The full-length CUX1 protein can promote DNA base repair and is related to tumor resistance.[43] The short subtype of CUX1 can regulate tumors through signaling pathways, such as PI3K/AKT, Wnt/β-catenin, and TGF-β.[44] MicroRNA target pathway network analysis in this study showed that the enriched pathways included the MAPK signaling pathway, PI3K-Akt signaling pathway, p53 signaling pathway, and mTOR signaling pathway. MAPK is an important transmitter of signals from the cell surface to the nucleus.[45] DEK targets the bronchial EMT-mediated asthma airway remodeling by inhibiting the MAPK signaling pathway.[46] PI3K-Akt signaling pathway has the effect of regulating cell growth, migration, proliferation, and metabolism. It is an important way to regulate airway cells in asthma patients, especially in the repair of lower respiratory tract epithelial cells and alleviating airway remodeling in asthma patients.[47] The p53 signaling pathway is an important way to regulate tumor cell senescence, and it plays an important role in the treatment of tumors.[48] In addition, when the lungs are infected by mycobacterium tuberculosis, the activated p53 signaling pathway can also regulate the body's innate immune response.[49] mTOR is involved in cell growth and proliferation. The mTOR pathway is regulated by various cell signals, including mitotic growth factors, hormones, and stress conditions. Activating PI3K/Akt/mTOR signaling inhibits autophagy in allergic asthma.[50]

This study has some limitations. For example, the experimental animals were limited to female mice. Moreover, the sample size of our study was small. Further studies are warranted.

This study demonstrated the multi-target, multi-pathway, and multifunctional features in the pathogenesis of bronchial asthma airway remodeling through comprehensive analysis. The pathogenesis of airway remodeling involved targets such as Tnrc6b, Sesn3, Baz2a, and Cux1. Signal pathways such as the MAPK signaling pathway, PI3K-Akt signaling pathway, p53 signaling pathway, and mTOR signaling pathway were also involved in asthma airway remodeling. This article may provide a better understanding of asthma and the potential for future treatments.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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