Circulating miRNA Profiles Associated with Lung Function Decline

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Research

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Abstract

Background: Lung function decline and miRNAs are known to involve in the pathogenesis process of respiratory diseases. However, the association between miRNA profiles and lung function decline was rarely elucidated. This study aimed to compare the expression pattern of plasma miRNAs among people with rapid lung function decline and healthy controls.

Methods: Ten controls and 10 cases with rapid lung function decline in last 3 years were enrolled from the Wuhan-Zhuhai cohort. The miRNA sequencing and bioinformatics analysis were carried out to explore miRNAs expression and possible signal pathways. Pearson correlation analysis was carried out to assess the associations between lung function and miRNAs expression levels.

Results: A total of 1209 detected miRNAs were differently expression in the reaseach participants. Among them, 17 miRNAs (miR-6749-5p, miR-6797-3p, miR-4468, miR-4301, miR-629-3p, miR-4713-3p, miR-486-3p, miR-450a-1-3p, miR-4732-5p, miR-514a-5p, miR-193b-5p, miR-6749-5p, miR-3168, miR-4691-5p, miR-6730-5p, miR-184, miR-486-5p) were significantly down-regulated in cases with reduced lung function. The expression levels of these miRNAs, except miR-514a-5p and miR-3168, were significantly correlated with lung function parameters. Through bioinformatics analysis, the Wnt signaling pathway, FoxO signaling pathway, MAPK signaling pathway, Pathway in cancer, inflammatory mediator regulation of TRP channels, and TGF-β signaling pathway maybe involved in mediating the association between 17 identified miRNAs and lung function decline.

Conclusions: The plasma miRNA profile of people with rapid lung function decline are different when compared with healthy controls. These miRNAs might become research candidates or biomarkers in the early progression of respiratory diseases.

1. Introduction

Respiratory diseases with poor outcomes of lower respiratory tract infections, chronic obstructive pulmonary disease (COPD), asthma, and lung cancer resulted in a large health burden and consistently rank the second leading cause of death worldwide [1, 2]. Pulmonary function test is an objective and effective method to assess the healthy status of respiratory system [3]. Little lung function decline is a normal feature of aging, but excessive decline during adulthood may lead to persistent airflow obstruction and is a common predictor for the development of respiratory diseases, including COPD, asthma, and cystic fibrosis [4–6]. Previous published study already found that rapid forced expiratory volume in 1s (FEV_1) decline was independently associated with both COPD-related hospitalizations and all-cause deaths [7]. The risk of rapid lung function decline for COPD-related hospitalizations was highest among people with normal FEV_1/ forced vital capacity (FVC) (≥ 70%), which indicated that the pathogenesis of COPD may begin much earlier as people developed some pulmonary abnormalities, like, FEV_1 declines more than 60 mL per year or FEV_1/FVC less than normal level [7, 8]. Unfortunately, the

underlying mechanisms for lung function decline in the pathological process of respiratory diseases remain to be fully elucidated.

MicroRNA (miRNA) is a kind of small non-coding RNAs, which plays an important role in regulating gene expression by forming complementary base pairs within the 3' untranslated regions of targeted mRNAs, participating in cell proliferation, differentiation, survival, and apoptosis [9]. Considering the fact that one miRNA could regulate up to hundreds of target genes, growing evidences demonstrated that miRNAs were involved in diverse biologic pathways, gene-disease interactions, and some diseases including respiratory diseases [10–12]. In 2018, Conickx and colleagues revealed that miR-218-5p was significantly down-regulated in COPD patients and strongly correlated with lung function decline [13]. Besides, Zhang and colleagues found that overexpressed miR-486-5p might enhance the Toll-like receptor 4 triggered inflammatory responses in COPD patients by targeting the HAT1 gene [14]. After Profiling the expression of miRNAs in 10 idiopathic pulmonary fibrosis (IPF) patients, Kusum and colleagues reported that let-7d was significantly reduced in IPF patients and positively correlated with pulmonary functions [15]. However, most of these studies investigated the association between miRNA profiles and lung function in serious patients, but not in early stage of respiratory diseases or normal subjects with slight injury.

Since plasma microRNAs were proved to be a kind of noninvasive biomarker reflecting pulmonary health [16], we conducted the present study to compare the expression pattern of plasma miRNAs among people with rapid lung function decline and healthy controls. Pulmonary parameter FEV_1/FVC% under 80% and FEV_1 declined more than 180 ml in last three years were regarded as rapid lung function decline. The plasma miRNA sequencing and bioinformatics analysis were carried out to explore miRNAs expression and possible signal pathways.

2. Research Design And Methods

2.1. Study population

The study participants were derived from the Wuhan-Zhuhai cohort, which has been described in detail elsewhere[17]. Briefly, a total of 4812 Wuhan or Zhuhai residents were recruited from 2011 to 2012, with follow-up every three years. The participants who completed the investigation in both 2014 and 2017 and never smoking were considered for this study. Cases with rapid lung function decline in this study were defined as: FEV_1/FVC% in 2014 was greater than 80% and FEV_1/FVC% in 2017 was less than 80%, and FEV_1 decreased by more than 180 mL. The control group is defined as those whose FEV_1/FVC% exceeded 80% in both 2014 and 2017, and the FEV1 decreased less than 180 mL after matching age, gender, and BMI (body mass index). As a result, a total of 10 pairs of case-control were randomly enrolled in this study. A standardized questionnaire was used to collect demographic characteristics, including age, gender, passive smoking status, alcohol consumption, physical activity, and family income by trained investigators. Anthropometric data including weight, height, and physical examination were measured by specialists. All participants in this study gave written, informed consent and the study was approved by

the Ethics and Human Subject Committee of Tongji Medical College, Huazhong University of Science and Technology.

2.2 Pulmonary function tests

Lung function test was conducted by a specialist using the electronic spirometer (Chestgraph HI-101, CHEST Ltd., Japan) according to the American Thoracic Society as described previously [18]. Three acceptable volume-time curves of lung function parameters, including FVC, FEV_1, maximal mid-expiratory flow, and peak expiratory flow were obtained.

2.3 Sample collection and RNA isolation

Approximately 5 mL fasting venous blood was collected into an EDTA tube for each person according to the standard method. Blood samples were centrifuged at 1500x g for 10 minutes at room temperature to spin down the blood cells. The supernatant plasma was isolated and stored at -80°C until analysis.

Total RNAs were extracted and purified from 500 μL plasma samples using the miRNeasy Plasma Kit (Qiagen, Hilden, Germany), as described in the manufacturer's protocol. QIAseq miRNA Library QC spike-ins (Qiagen, Hilden, Germany) were added during the process of RNA extraction to provide external controls for the quality of the RNA isolation, library preparation, and sequencing analysis.

2.4 miRNA profiling and qPCR quality

The integrity and concentration of purified RNA were analyzed with an Agilent Bioanalyzer 2100 before library preparation. Then acceptable RNAs were used as a template for the QIAseq miRNA library kit (Qiagen, Hilden, Germany), which ligated specific adapters to the 3' and 5' sides of miRNA, reversely transcripted miRNAs to cDNA, and processed library amplification. High-sensitivity DNA chips and Illumina HiSeq 2500 instrument (Illumina, CA, USA) were conducted to assess the quality of the libraries and generate sequencing reads, respectively. The results of sequencing reads were validated by RT-PCR according to the QIAseq miRNA Library QC PCR panel kit (Qiagen, Hilden, Germany). U6 RNA was regarded as the reference gene to normalize the expression of five plasma miRNAs in RT-PCR and RNA sequencing. All procedures for next-generation sequencing (NGS) were performed by CapitalBio Technology (Beijing, China).

2.5 Bioinformatics analysis

Three miRNA target databases, including Miranda (http://mirdb.org/miRDB/), TargetScan (http://www.targetscan.org/), and miRPathDB (http://mpd.bioinf.uni-sb.de/) were used to predict the target genes of miRNAs associated with lung function decline. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was carried out to analyze those target genes which were associated with at least two miRNAs in three databases.

2.6 Statistical analysis

Continuous variables were shown as the mean ± standard deviation and categorical variables were expressed as a number and percentage. Chi-square test and Student's t-test were respectively carried out to compare categorical and continuous characteristics between cases and controls. The row data of each miRNA sequencing were first processed and mapped with human genome version hg19 using bowtie software. Only miRNAs with expression levels of > 1 counts per million (CPM) in more than half of the samples were considered detectable miRNAs and then normalized by "EdgeR" package to identify differentially expressed miRNAs between two groups. Pearson correlation analysis was carried out to assess the correlation between log-transformed CPM by sequencing and CT value by qPCR, as well as associations between lung function and miRNAs expression levels. A false discovery rate (FDR) adjusted *P*-value of < 0.10 was considered statistically significant. All statistical analyses were conducted using SAS 9.4 (SAS Institute, NC, USA) and R version 3.4.3 (R core team, 2016).

3. Result

3.1 Basic characteristics

The basic characteristics and lung function of 20 individuals was presented in Table 1. In this study, the most of the participants were female, accounting for 90% of the objects, and the mean age of the cases and controls were 58.0 and 57.8 years old respectively. There were no differences noted with among demographic characteristics, including age, height, weight, BMI, physical activity, passive smoking, drinking, and income between case and control groups. In 2014, the lung function levels of the cases (FEV_1 was 2.65 ± 0.43 L, FVC was 2.28 ± 0.35 L, FEV_1/FVC% was 85.3 ± 4.5%) and controls (2.69 ± 0.51 L, 2.25 ± 0.43 L, 83.7 ± 2.5 %) were at the same level (all $P > 0.05$). While in 2017, FEV_1 and FEV_1/FVC% for cases (1.91 ± 0.29 L, 74.2 ± 2.4 %) were significantly lower than those in controls (2.38 ± 0.43 L, 86.1 ± 3.7 %) ($P < 0.05$). The reduced FEV_1 and FEV_1/FVC% of the case group (0.37 ± 0.21 L, 11.9 ± 6.7 %) were significantly higher than that of the control group (-0.13 ± 0.08 L, -2.4 ± 4.4 %) ($P < 0.05$).

Table 1
Basic characteristics of cases with lung function decline and healthy controls.

Characteristics	control (n = 10)	case (n = 10)	*P* value
Male, n (%)	1 (10%)	1 (10%)	1
Age, year (mean ± SD)	58.0 ± 7.7	57.8 ± 6.3	0.943
Height, cm (mean ± SD)	157.8 ± 8.0	156.4 ± 3.1	0.594
Weight, kg (mean ± SD)	59.2 ± 9.0	56.8 ± 10.3	0.588
BMI, kg/m^2 (mean ± SD)	23.8 ± 3.3	23.2 ± 3.9	0.721
Regular physical activity, n (%)	4 (40%)	3 (30%)	0.639
Passive smoking (n, %)	3 (30%)	3 (30%)	1
Current drinking (n, %)	1 (10%)	2 (20%)	0.531
Income > 30,000 RMB/year, n (%)	4 (40%)	6 (60%)	0.371
Lung function in 2014			
FVC, L (mean ± SD)	2.69 ± 0.51	2.65 ± 0.43	0.87
FEV_1, L (mean ± SD)	2.25 ± 0.43	2.28 ± 0.35	0.857
FEV_1/FVC, % (mean ± SD)	83.7 ± 2.5	86.1 ± 5.4	0.218
Lung function in 2017			
FVC, L (mean ± SD)	2.78 ± 0.58	2.57 ± 0.42	0.371
FEV_1, L (mean ± SD)	2.38 ± 0.43	1.91 ± 0.29	0.01
FEV_1/FVC, % (mean ± SD)	86.1 ± 3.67	74.2 ± 2.41	< 0.001
Lung function decline in three years			
FVC, L (mean ± SD)	-0.09 ± 0.13	0.08 ± 0.15	0.011
FEV_1, L (mean ± SD)	-0.13 ± 0.08	0.37 ± 0.21	< 0.001
FEV_1/FVC, % (mean ± SD)	-2.4 ± 4.4	11.9 ± 6.7	< 0.001
Abbreviations: BMI, body mass index, FVC, forced vital capacity, FEV_1, forced expiratory volume in 1s.			

3.2 miRNAs expression profiles

After processing the data of RNA sequencing, the composition of detected small RNAs (sRNA) was classified into different categories, including miRNA, piRNA, rRNA, tRNA, mRNA, novel RNA, and others

(Fig. 1). The proportions of each kind of sRNA were similar in case and control groups, in which miRNAs made up more than half of the sRNA (68.9% in the case group and 57.4% in the control group). mRNA accounted for the least in both case and control groups, while tRNA was lower in cases than in controls but not statistically different. In general, all kinds of sRNAs were not significantly different between the two groups.

A total of 1209 miRNAs were efficiently detected in plasma from the research participants. Compared with the expression levels in healthy controls, 8 up-regulated and 81 down-regulated miRNAs were identified to differently expressing in the case group with an absolute $\log_2$ (Fold change) > 1.069 (1 unit of standard deviation) and *P*-value < 0.05 (Fig. 2). Notably, there were 17 miRNAs with FDR lower than 0.1 that were considered as significantly differential expressed miRNAs, as shown in Table 2, all of which had lower expression in the cases than in the controls. Additional 24 miRNAs showed an $0.10 \leq \text{FDR} < 0.2$ in Table S1, including 23 down-regulated and 1 up-regulated miRNA.

Table 2
Top 17 significantly differential expressed miRNAs between cases with lung function decline and controls.

miRNAs	Fold change	*P* value	FDR
miR-6749-5p	-5.26	3.3E-08	4.0E-05
miR-6797-3p	-3.18	1.8E-06	0.0008
miR-4468	-4.84	1.9E-06	0.0008
miR-4301	-3.33	6.1E-06	0.0018
miR-629-3p	-1.72	7.7E-06	0.0019
miR-4713-3p	-2.94	4.7E-05	0.0086
miR-486-3p	-2.73	5.0E-05	0.0086
miR-450a-1-3p	-3.83	8.8E-05	0.0133
miR-4732-5p	-2.75	0.0001	0.0166
miR-514a-5p	-2.85	0.0004	0.0425
miR-193b-5p	-1.96	0.0004	0.0436
miR-6747-5p	-2.48	0.0004	0.0446
miR-3168	-2.59	0.0007	0.0619
miR-4691-5p	-2.31	0.0007	0.0636
miR-6730-5p	-2.13	0.0008	0.0643
miR-184	-2.08	0.0009	0.0657
miR-486-5p	-1.62	0.0011	0.0772

Fold change = Log_2 (case/control).

Besides, 5 plasma miRNAs, including miR-23a-3p, miR-30c-5p, miR-103a-3p, miR-191-5p, and miR-451a, were examined to validate the sequencing results by quantitative RT-PCR (Figure S1). Results demonstrated that the expression levels of miRNAs using miRNA profiles were highly consistent with those derived from RT-PCR, with correlation coefficients ranged from 0.88 to 0.97.

3.3 Relationship between plasma miRNA and lung function

We then investigated whether there were associations between the expression levels of 17 plasma miRNAs and lung function. As shown in Table 3, the most of the plasma miRNAs, except miR-514a-5p and miR-3168, were significantly correlated with FEV_1/FVC% in 2017 with highly correlation coefficients ($r \geq 0.88$). Similar correlations were also found between the expression levels of miRNAs and FEV_1/FVC% decline in three years. In addition, miR-6749-5p, miR-6797-3p, miR-4468, miR-4301, miR-629-3p, miR-4713-

3p, miR-6747-5p, miR-4691-5p, and miR-184 showed a positive correlation with the change of FEV_1 in three years (r range from 0.459 to 0.675, $P < 0.05$).

Table 3
Correlation between miRNAs and lung function parameters.

miRNAs		lung function in 2017			lung function changes in 3 years		
		FVC	FEV_1	FEV_1/FVC%	FVC	FEV_1	FEV_1/FVC %
miR-6749-5p	r	-0.303	-0.025	**0.676**	-0.103	**0.485**	**0.682**
	P	0.194	0.915	**0.001**	0.666	**0.030**	**0.001**
miR-6797-3p	r	-0.163	0.117	**0.720**	0.105	**0.580**	**0.708**
	P	0.492	0.623	**0.000**	0.659	**0.007**	**0.000**
miR-4468	r	-0.164	0.163	**0.796**	0.136	**0.668**	**0.768**
	P	0.489	0.491	**0.000**	0.567	**0.001**	**0.000**
miR-4301	r	-0.223	0.023	**0.617**	-0.084	**0.459**	**0.654**
	P	0.344	0.923	**0.004**	0.726	**0.042**	**0.002**
miR-629-3p	r	-0.131	0.211	**0.855**	0.316	**0.675**	**0.682**
	P	0.582	0.371	**0.000**	0.175	**0.001**	**0.001**
miR-4713-3p	r	-0.005	0.229	**0.610**	-0.023	**0.539**	**0.750**
	P	0.984	0.332	**0.004**	0.922	**0.014**	**0.000**
miR-486-3p	r	-0.132	0.097	**0.580**	-0.099	0.396	**0.585**
	P	0.580	0.683	**0.007**	0.678	0.084	**0.007**
miR-450a-1-3p	r	-0.248	-0.058	**0.474**	-0.176	0.274	**0.467**
	P	0.292	0.808	**0.035**	0.458	0.242	**0.038**
miR-4732-5p	r	-0.201	0.014	**0.504**	-0.207	0.342	**0.551**
	P	0.395	0.953	**0.023**	0.382	0.140	**0.012**
miR-514a-5p	r	-0.179	-0.008	0.383	-0.142	0.246	0.388
	P	0.451	0.973	0.096	0.549	0.296	0.091
miR-193b-5p	r	-0.237	-0.003	**0.562**	-0.187	0.356	**0.562**
	P	0.315	0.991	**0.010**	0.431	0.124	**0.010**
miR-6747-5p	r	-0.224	0.071	**0.700**	-0.070	**0.488**	**0.656**
	P	0.341	0.765	**0.001**	0.769	**0.029**	**0.002**
miR-3168	r	-0.031	0.049	0.224	-0.053	0.168	0.276
	P	0.898	0.836	0.342	0.824	0.479	0.239

miR-4691-5p	r	-0.034	0.208	**0.608**	0.089	**0.604**	**0.728**
	P	0.887	0.380	**0.004**	0.710	**0.005**	**0.000**
miR-6730-5p	r	-0.266	-0.025	**0.566**	-0.153	0.405	**0.600**
	P	0.257	0.915	**0.009**	0.519	0.077	**0.005**
miR-184	r	-0.122	0.097	**0.557**	0.061	**0.537**	**0.679**
	P	0.607	0.685	**0.011**	0.797	**0.015**	**0.001**
miR-486-5p	r	-0.125	0.096	**0.559**	-0.124	0.374	**0.572**
	P	0.600	0.687	**0.010**	0.603	0.104	**0.008**

3.4 Enrichment biological function analysis

To explore the potential biological mechanisms underlying the role of these 17 differential expressed miRNAs in lung function decline, we performed targeted gene prediction and pathway analysis. A total of 5351 unique genes were predicted to have interaction with these miRNAs in three databases. After the targeted genes were filtered for high-confidence predictions and predicted frequency, 1379 target genes were involved in the following KEGG signaling pathway analysis. As presented in Fig. 3, those target genes were mainly enriched in 6 potential pathways, including the Wnt signaling pathway, FoxO signaling pathway, MAPK signaling pathway, Pathway in cancer, inflammatory mediator regulation of TRP channels, and TGF-β signaling pathway.

4. Discussion

In the present study, we conducted RNA sequencing to explore the profile of plasma miRNAs in people with rapid lung function decline. Using the NGS miRNA platform, a total of 1209 miRNAs was identified and 17 of them were significantly down-regulated in cases with lung function decline when compared with controls. In addition, the expression levels of these 17 miRNAs, except miR-514a-5p and miR-3168, were found to be significantly correlated with lung function parameters. Bioinformatics analyses predicted that these 17 miRNAs were mainly enriched in 6 associated pathways, including the Wnt signaling pathway, FoxO signaling pathway, MAPK signaling pathway, Pathway in cancer, inflammatory mediator regulation of TRP channels, and TGF-β signaling pathway.

To our knowledge, this is the first study to profile the expression pattern of plasma miRNAs associated with reduced lung function. Lung function, a vital indicator for the evaluation of respiratory health, is widely used in the clinical diagnosis because of its simplicity and reproducibility [19]. Recent studies revealed that dysregulated miRNAs were involved in the pathological process of respiratory disesases due to the role of multiple gene regulation in apoptosis, autophagy, cellular differentiation, endothelial cell proliferation, and airway inflammation [20, 21]. Most of these studies focused on patients who already

suffered from respiratory diseases, but not people with normal lung function at baseline [22–25]. Therefore, it is necessary to evaluate the association between different miRNAs expression profiles and lung function decline in early stage. Accordingly, we found 17 miRNAs significantly down-regulated in people with rapid lung function decline. In this study, we selected cases with rapid lung function decline through a cohort that was followed for 3 years. Both cases and controls had lung function paramaters before and after 3 years. At the same time, in order to avoid direct interference of smoking on miRNA, the study participants excluded cigarette smokers. Therefore, our results could better observe the specific miRNAs related to lung function decline.

Of 17 differential expressed miRNAs identified in this study, miR-629-3p was previously reported to be significantly down-regulated in plasma from COPD patients with a fold change of 0.38 when compared with healthy controls [26]. Maes and colleagues reported that miR-629-3p influences the pathogenesis of neutrophilic asthma through inducing epithelial expression of IL-8 and IL-6 [27]. The finding indicated that miR-629-3p may involve in the complex inflammation feedback loop of IL-6/IL-6 receptor/signal transducer and activator of transcription 3 signaling. While, up-regulation of miR-629 was observed in various cancers, including hepatocellular carcinoma, renal cell carcinoma, pancreatic and laryngeal cancer [28–31]. Epithelial-mesenchymal transition (EMT), which plays an essential role in the pathological process of respiratory diseases, is the common target for tumor-promotive or pulmonary fibrosis roles of miR-629. Liu and colleagues reported that miR-629 promoted the progression of non-small cell lung cancer by targeting FOXO1 and regulating EMT/PI3K/AKT pathways [32], and MYCT1/SP1/miR-629-3p/ESRP2 is another signaling pathway to inhibit the EMT [29]. Besides, miR-629 directly targeted TRIM33, which inhibits the TGF-β/Smad signaling pathway, could enhance the expression of EMT-related factors [31]. Therefore, inflammation and EMT are expected to serve as a bridge connecting miR-629-3p and lung function decline in further research.

miR-486 is a highly conserved miRNA in mammals, which is located at Chr:8p11 and processed into two mature miRNAs: miR-486-5p and miR-486-3p. A previous study by Ji and colleagues reported that decreased miR-486-5p expression was linked to the progression of pulmonary fibrosis in both humans and mice [33]. Over-expression of miR-486-5p could attenuate pulmonary fibrosis and repress TGF-β1-induced fibrogenesis, which was proved in non-small cell lung cancer and renal cell carcinoma [34, 35]. These findings demonstrated that miR-486-5p has a strong anti-fibrotic activity in lung tissues. Interestingly, the protective role of miR-486-5p was also found in human lung alveolar epithelial cells exposed to PM2.5 [36]. miR-486-5p may play role through PTEN and FOXO1 pathway which mediated the protective effects by reducing cell apoptosis and reactive oxygen species generation. Similarly, the study by Chai and colleagues concluded that miRNA-486-5p improved nucleus pulposus cell viability, and inhibited inflammation cytokines and ECM degradation partly via inhibition of FOXO1 expression [37]. Thus, down-regulation of miR-486 may be one of the regulators which mediated the decline of lung function and the detailed mechanism needs more research in the future.

miR-184 was also reported to play a protective role in lung tissue [38]. Using microRNA expression array among 25 healthy and IPF patients, miR-184 expression levels was down-regulated in IPF patient tissues. Further *in vitro* study investigated that miR-184 could bind to the smad2- and akt-3'UTRs and repress the downstream signaling pathways triggered by TGF-β [38]. However, Cristina and colleagues reported contradictory results that miR-184 acted a downstream effector of albuminuria through LPP3 to promote tubulointerstitial fibrosis [39]. This difference may be attributed to the type 2 diabetes rat model used by Christina, who studied the kidneys in the late stage of the disease. Further research is needed to investigate whether fibrosis is involved in the association between miR-184 and lung function decline. The other miRNAs we found in this study have not previously been reported and were novel objects to study the association with respiratory diseases in the future.

To gain insight into the potential functional importance of these 17 miRNAs, we analyzed the signaling pathways with three databases. Most of the associated pathways have been revealed to be involved in the pathological process of respiratory diseases. The developmental WNT pathway is fundamental for lung development, and altered WNT activity has been reported to contribute to lung epithelium injury repair [40]. FOXO transcriptional factors, including FOXO1, FOXO3, were active during bacterial infection in respiratory epithelium and enhancing various epithelial innate immune functions [41]. MAPKs are serine-threonine kinases that widely mediate intracellular signaling associated with cell survival, proliferation, differentiation, apoptosis, and transformation [42]. Air pollutants, lipid mediators, infection, stresses, and extracellular cytokines could activate the p38 MAPK pathway and might therefore contribute to inflammation gene expression [42]. Activating of JNK pathway, another member of MPAKs family, was also reported to participate in inflammatory response by aggravating oxidative stress in lung tissue [43]. The inflammatory mediator regulation refers to PGE2, bradykinin, ATP, and pro-inflammatory cytokines that are generated during acute lung tissue injury [44]. As previously mentioned, TGF-β is crucial in pulmonary fibrosis and emphysema via mediating fibroblast activation, ECM synthesis, and immune response [45]. Further researches are needed to reveal whether these associated signaling pathways were underlying the pathogenesis of lung function decline and the early stage of respiratory diseases including COPD, asthma and pulmonary fibrosis.

Our study have some strengths. Firstly, this study was based on a cohort study and participants enrolled were follow-up in three years. Then, we used NGS technology in miRNAs profiling to provide an open and objective result. However, some limitations of our study need to be considered. First, the number of participants enrolled in this study is relatively small, and further studies with a large population are needed to validate our findings. Second, the expressing levels of miRNAs we identified were not all proved by RT-PCR. To validate the sequencing results, we measured the expression of 5 endogenous miRNAs by RT-PCR and found a high correlation ($R^2 \geq 0.88$) with sequencing results. Third, it's hard to determine the true origin of miRNAs we identified in plasma. But according to The Human miRNA Tissue Atlas (website https://ccb-web.cs.uni-saarland.de/tissueatlas/) [46], most miRNAs we identified were expressed in lung tissue.

5. Conclusion

Our results suggest that several plasma miRNAs associated with lung function decline, which might become research candidates or biomarkers in the early progression of various respiratory diseases. Further studies are encouraged to validate our findings in more robust studies.

6. Abbreviations

chronic obstructive pulmonary disease (COPD), forced expiratory volume in 1s (FEV1), forced vital capacity (FVC), MicroRNA (miRNA), idiopathic pulmonary fibrosis (IPF), next-generation sequencing (NGS), Kyoto Encyclopedia of Genes and Genomes (KEGG), counts per million (CPM), false discovery rate (FDR), small RNAs (sRNA), Epithelial-mesenchymal transition (EMT).

7. Declarations

Ethics approval and consent to participate

The study was approved by the Ethics and Human Subject committee of Tongji Medical College, and the approval ID was [2016] IEC S128. Written informed consent was obtained from all participants.

Consent for publication

No applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Competing interests

All authors declared that they have no competing interests.

Authors's contributions

Man Cheng researched and wrote this manuscript, Yang Xiao, Zi Ye, and Linglin Yu participated in data analysis, Bin Wang, Xiaobin Feng, and Ge Mu participated in writing, Jixuan Ma and Weihong Cheng contributed to project administration. All authors read and approved the final manuscript.

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Figures

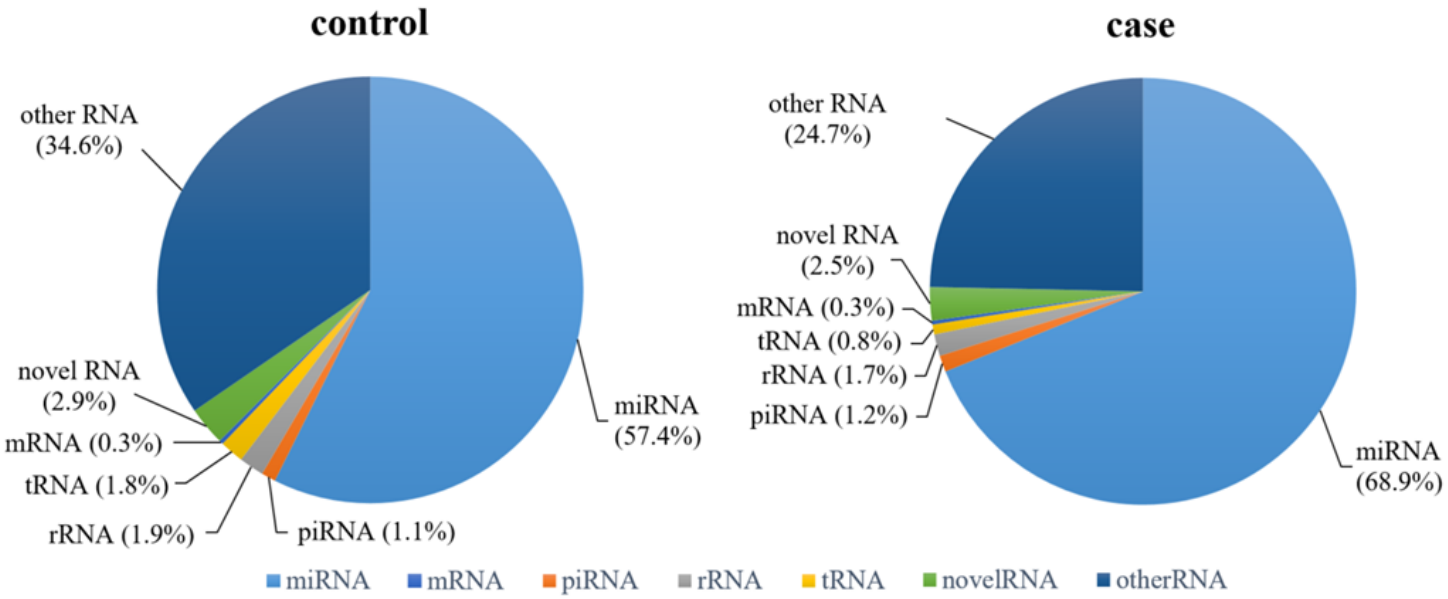

Figure 1

The proportions of different kinds of small RNAs.

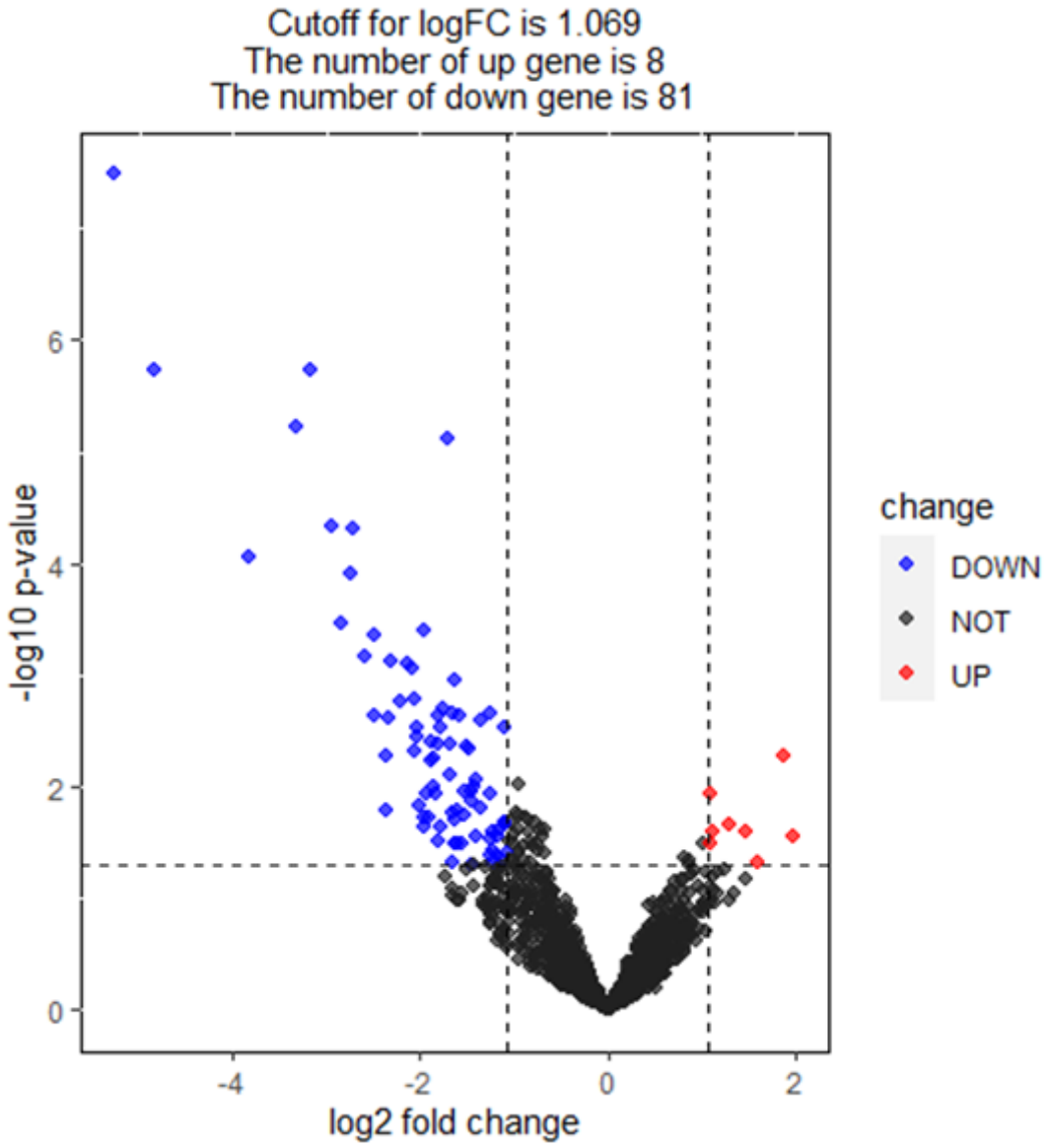

Figure 2

The volcano plot for the 1209 miRNAs up- and down-regulated in cases with lung function decline compared with controls. The X-axis is log2 fold-change and the Y-axis is log10 P-value.

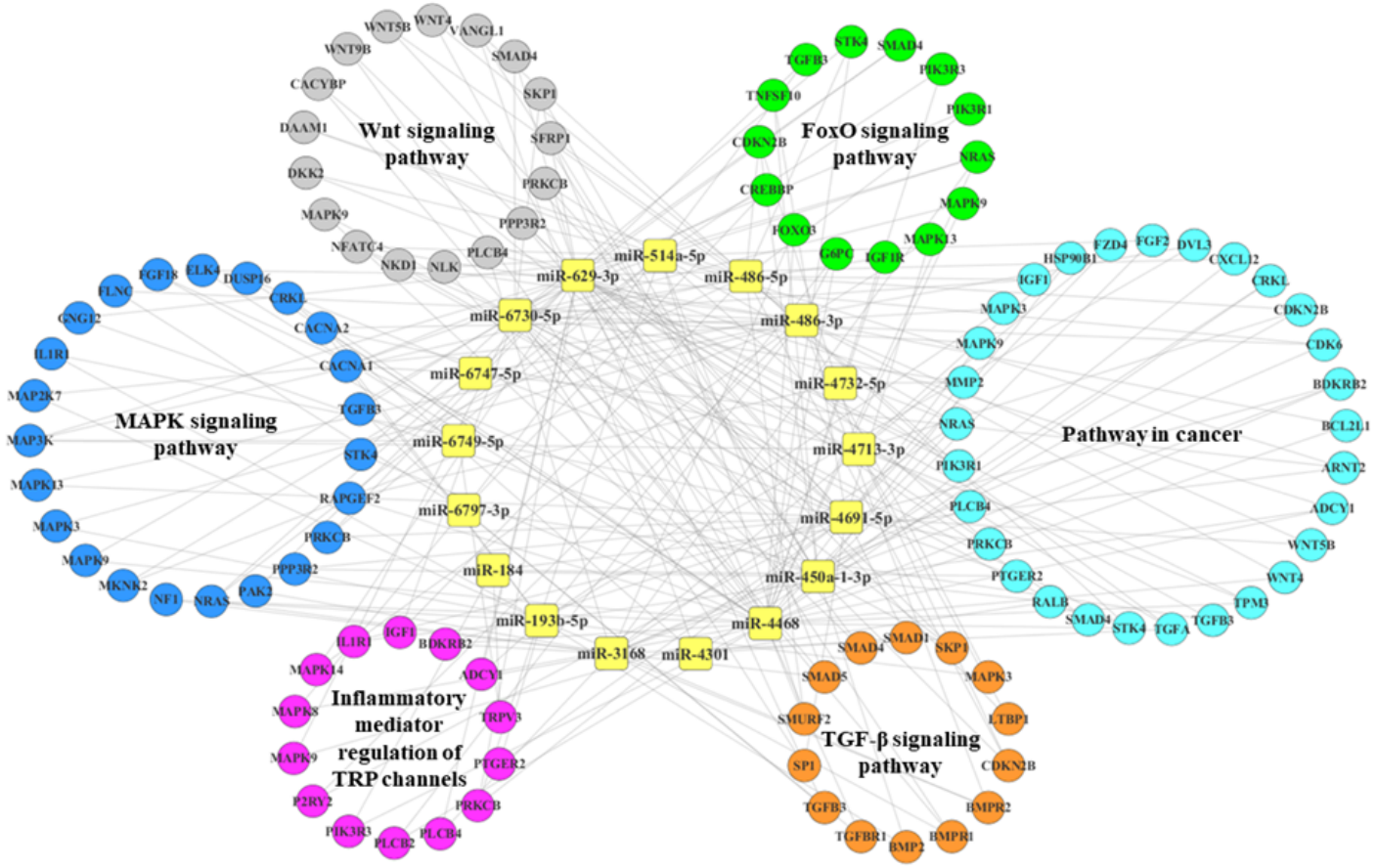

Figure 3

Enrichment of KEGG pathways and target genes of 17 significantly differential expressed miRNAs.

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