Int J Clin Exp Pathol 2017;10(4):4860-4865
www.ijcep.com /ISSN:1936-2625/IJCEP0041532

Original Article

MicroRNA expression profiles of whole blood in chronic obstructive pulmonary disease

Yipeng Ding[1*], Zhongjie Tian[1*], Hua Yang[2], Hongxia Yao[1], Ping He[1], Yanhong Ouyang[1], Jinjian Yao[1], Min Li[1], Tianbo Jin[2]

[1]Department of Emergency, Hainan General Hospital, Haikou, Hainan, China; [2]School of Life Sciences, Northwest University, Xi'an, China. [*]Equal contributors.

Received October 10, 2016; Accepted November 25, 2016; Epub April 1, 2017; Published April 15, 2017

Abstract: Purpose: Chronic obstructive pulmonary disease (COPD) is a major and an increasingly prevalent health problem worldwide. Studies have shown that MicroRNA involved in the prevention and treatment of respiratory diseases. MicroRNA upregulation and downregulation effectively regulate the biological processes of these diseases. Little is known about the microRNA (miRNA) expression of COPD. Identifying expression patterns of miRNAs in COPD may enhance our understanding of the mechanisms of disease. Methods: We selected 72 samples and measured the expression of 13 miRNAs using quantitative reverse-transcription (RT) polymerase chain reaction (PCR). Results: Between patients with COPD and healthy control subjects, we found the expression of seven miRNAs was significantly in Li population ($P < 0.05$). We did not found miRNAs were under-expressed in COPD patients of Li population. In Han population there have only one miRNA (hsa-miR-196b-5p) were under-expressed of COPD patients. We analyzed the miRNA expression of the patients with COPD and health controls for differentially expressed miRNAs in smokers versus nonsmokers. We have not found any miRNAs were significantly. Conclusion: Our findings showed miRNA dysregulation in COPD and its relation to established molecular backgrounds. These findings may reveal important insights into the pathogenesis of COPD.

Keywords: Chronic obstructive pulmonary disease (COPD), RT-PCR, microRNAs, smoking

Introduction

Chronic obstructive pulmonary disease (COPD) is an airflow obstruction disease, and it is caused by chronic bronchitis, emphysema, and/or disease of small airways [1]. It can caused the significant morbidity and mortality in the worldwide, leading to an important socio-economical burden [2]. MiRNAs are a growing class of noncoding and endogenous RNAs that function mostly as translational repression, cleavage, or destabilization of the target [3, 4]. MiRNAs have been participate in the pathogenesis of various malignancies [5], cardiovascular [6], endocrine [7] and neurological diseases [8]. Recent studies have described a role for certain miRNAs in asthma and lung fibrosis [9, 10]. To date, however, there is little understanding of the role of miRNAs in COPD.

Our goal in this study was to determine if miRNAs were differentially expressed in of patients with COPD and if miRNA expression may be linked to mRNA expression and thus biological pathways relevant to the pathogenesis of COPD.

Materials and methods

Study participants

In all, 72 subjects from People's Hospital of Hainan Province were included in this study. All participants were Li and Han population of Hainan province. Participants were classified into 4 groups: never smokers without COPD, smokers without COPD, never smokers with COPD, and smokers with COPD. COPD was newly diagnosed according to the criteria established by the NHLBI/WHO Global Initiative for COPD (GOLD) [11]. The entry criteria for COPD cases were post-bronchodilator forced expiratory volume in 1 second (FEV1) < 80% predicted and FEV1/FVC (forced vital capacity) < 0.7. Finally, we chose 36 patients with COPD. Smo-

Table 1. Characteristics of COPD patients and control participants

		Cases (n = 36)	Controls (n = 36)
		N	N
Sex	Female	12	12
	Male	6	6
Nationality	Han	18	18
	Li	18	18
Smoking Status	Non-smoker	21	22
	Smoker	15	14

kers were defined as smokers that still smoked at the moment of participation in the study or had quit smoking for less than one year prior to their participation in the study. Additional, random selected 36 unrelated healthy individuals were taken as control group; all of the controls were healthy without chronic diseases and related to the vital organs. The study was approved by the Clinical Research Ethics Committees of People's Hospital of Hainan Province.

RNA extraction

We used the TRIzol (Invitrogen) and miRN easy mini kit (QIAGEN) isolated Total RNA and according to manufacturer's instructions. And we efficiently recovered all RNA species, including miRNAs. RNA quality and quantity was measured by using nanodrop spectrophotometer (ND-1000, Nanodrop Technologies) and RNA Integrity was determined by gel electrophoresis.

Quantitative PCR (Q-PCR)

Q-PCR was carried out in duplicate using 250 ng aliquots of total RNA. The RNA was converted into cDNA using the ABI High-Capacity cDNA Archive Kit according to manufacturer's instructions. 2 μl cDNA was reacted with 2*miR cute miRNA Premix in 10 μl reaction including 0.4 μl of the appropriate forward and reverse primers and 7.2 μl ddH_2O. Thermal cycling was carried out on an ABI 7500 Sequence Detection System (Applied Biosystems, Warrington, UK) with the following profile 50°C for 10 minutes, 94°C for 10 minutes, 94°C for 10 seconds 60°C for 10 seconds and 72°C for 34 seconds for 45 cycles. Quantitation was relative to a standard curve (genomic DNA) according to manufacturer's instructions. Data were normalized to a combination house keeper gene which was a weighted mean of data from β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and cyclophilin.

Data analysis

The gene expression levels were examined using Custom RT^2 Profiler™ PCR Array (SABiosciences) performed on Roche Light Cycler 480. Raw Ct values were normalized using cel-miR-39-3p as a "reference gene" and those Ct values greater than 40 were replaced with 40 before normalization. The $2^{-\Delta\Delta Ct}$ method and Student's t-test were used in the analyses of differentially expression genes. The $2^{-\Delta\Delta Ct}$ is the normalized gene expression 2-Average ΔCt in the test samples divided the normalized gene expression 2-Average ΔCt in the control samples. Fold-change values greater than 2 indicate a significant positive-or an up-regulation; fold-change values less than 0.5 indicate a significant negative or down-regulation. The *p* values were calculated based on a Student's t-test of the replicate $2^{-\Delta Ct}$ values for each gene in the control group and treatment groups, and *p* values less than 0.05 indicated statistically significant differences. All the calculations were performed with a tool of online analysis, RT^2 Profiler PCR Array Data Analysis version 3.5 (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).

Results

Characteristics of the 72 study subjects are shown in **Table 1**, including 20 COPD and 10 controls in Li population, 20 COPD and 10 controls in Han population. In addition, there were 30 current smoking patients with COPD and 20 ex-smoking patients with controls. In **Table 2**, we analyzed 13 miRNAs, and first compared the expression of with COPD and without COPD miRNAs in Li and Han population. Between patients with COPD and healthy control subjects, we found the expression of seven miRNAs was significantly in Li population ($P < 0.05$). And we found seven of these 13 miRNAs were upregulated (hsa-miR-146b-5p, hsa-miR-141-3p, hsa-miR-186-5p, hsa-miR-4532, hsa-miR-4635, hsa-miR-2681-3p, hsa-miR-4503) in COPD patients compared with controls in

Table 2. MicroRNAs differentially expressed in Li and Han Population

ID	Name	Li population			Han population		
		COPD vs. Control			COPD vs. Control		
		Up/Down	Fold Change	*P* Value	Up/Down	Fold Change	*P* Value
1	hsa-miR-146b-5p	up	25.7731	0.043735	-	0.5984	0.066248
2	hsa-miR-141-3p	up	587.6374	0.032303	-	0.0501	0.434735
3	hsa-miR-193a-3p	-	26.0125	0.667475	-	0.1069	0.55406
4	hsa-miR-186-5p	up	10.9463	0.042967	-	0.4492	0.054105
5	hsa-miR-101-3p	-	4.6094	0.057239	-	1.3492	0.313696
6	hsa-miR-576-5p	-	12.5441	0.05356	-	0.3931	0.143509
7	hsa-let-7i-3p	-	32.9136	0.272045	-	0.7611	0.441868
8	hsa-miR-196b-5p	-	21.3204	0.148745	down	0.0286	0.013267
9	hsa-miR-4532	up	48.9545	0.021159	-	0.2981	0.406835
10	hsa-miR-4635	up	46.9871	0.032839	-	0.8378	0.796565
11	hsa-miR-2681-3p	up	108.6548	0.02806	-	0.0927	0.107168
12	hsa-miR-4503	up	85.4654	0.034508	-	0.0868	0.072593
13	hsa-miR-4421	-	33.8393	0.180815	-	0.1112	0.100019

Table 3. MicroRNAs differentially expressed with smokers and without smokers in COPD and Controls

ID	Name	Control			COPD		
		Smoker vs. non-smoker			Smoker vs. non-smoker		
		Up/Down	Fold Change	*P* Value	Up/Down	Fold Change	*P* Value
1	hsa-miR-146b-5p	-	1.1139	0.593135	-	0.1598	0.400738
2	hsa-miR-141-3p	-	4.2017	0.949525	-	0.0368	0.611249
3	hsa-miR-193a-3p	-	1.2354	0.790662	-	0.5053	0.155578
4	hsa-miR-186-5p	-	2.4369	0.765701	-	0.2254	0.927511
5	hsa-miR-101-3p	-	1.3788	0.367135	-	0.9528	0.840689
6	hsa-miR-576-5p	-	1.3463	0.932517	-	0.4329	0.478337
7	hsa-let-7i-3p	-	1.4948	0.886754	-	0.4408	0.232764
8	hsa-miR-196b-5p	-	1.8258	0.972897	-	0.1218	0.774597
9	hsa-miR-4532	-	1.4766	0.881173	-	0.2505	0.924137
10	hsa-miR-4635	-	3.9868	0.83498	-	0.3399	0.758272
11	hsa-miR-2681-3p	-	2.1309	0.75592	-	0.1229	0.912281
12	hsa-miR-4503	-	1.3246	0.513512	-	0.1719	0.739537
13	hsa-miR-4421	-	1.6101	0.305497	-	0.1356	0.300282

Li population. We did not found miRNAs were underexpressed in COPD patients of Li population. In Han population there have only one miRNA (hsa-miR-196b-5p) were under expressed of COPD patients.

When focusing on the subgroup of between never smokers and current smokers with COPD and healthy controls the miRNA profiles. We analyzed the miRNA expression of the patients with COPD and health controls for differentially expressed miRNAs in smokers versus nonsmokers. We have not found any miRNAs were significantly (**Table 3**).

Discussion

MiRNAs have been shown in preclinical experiments to regulate many biological processes, including cell development, proliferation, differentiation and apoptosis [3, 12]. These effects occur through the suppressed expression of key target genes. MiRNAs regulate the expression of genes involved in biological processes relevant to the progression of chronic lung disease, including cellular stress, cell differentiation and apoptosis [13-15]. Despite the fact that much has been learned about the pathogenesis of this disease with systemic inflamma-

tory components [16], few studies until now have focused on the role of miRNAs in COPD. Here, in our study, we assessed the miRNA expr-ession patterns in COPD and different smok-ing status of Li and Han population. To the best of our knowledge, this is the first study to identify miRNA expression in COPD of Li and Han population. Our analysis identified seven miRNAs presented different degrees of expression between the two groups in Li population. On the other hand, in Han population we found one miRNA (hsa-miR-196b-5p) were under expressed of COPD patients. In addition, we stratified according to smoking status, but did not find any miRNAs were significantly.

miR-146b-5p is one of the largest associated with a variety of malignant tumors, and it is a small RNA transcription function independent of the earliest discovered [17]. miR-146b-5p is an important regulatory factor in inflammatory signaling pathways, ands recorded the MiRNAs differences in the proliferation, apoptosis, immune mechanism in the hematopoietic system [18]. Recently a large number of studies have shown that miR-146b-5p expression changes and the risk of cancer, the occurrence and metastasis of tumor, invasion ability have a great relevance [19]. Many studies have been reported function research about miR-146b-5p as a tumor prognostic molecular marker. Patnaik et al, Raponi et al and Patnaik et al [20-22] have found that miR-146b-5p high expression is associated with non-small cell lung cancer with poor prognosis, and miR-146b-5p high expression is index of small cell lung cancer recurrence. miR-146b-5p overexpression can also reduce the invasion and metastasis of tumor cells [18]. Other studies have been shown that miR-146b-5p can negative regulating NF-kB pathway [23]. In our study, we found the expression of miR-146b-5p miRNAs was significantly in Li population, miR-146b-5p may have close relationship with COPD of Li population.

Bostjancic et al [24] confirmed miR-186 participate in a variety of physiological and pathological process in cardiovascular disease. Studies have been shown that miR-186 expressed in tumor epithelial cells increased and decreased P2X7 mRNA level [25]. miR-186 has been shown as a proliferation inhibitor in lung adenocarcinomas, by targeting CCND1, CDK2, and CDK6 [26] and CCND1 has also been demonstrated as a predicted target of miR-26b [27]. Recent literature reported that miR-186 by inhibiting CyclinD1 (CCND1), cyclin dependent kinase (CDK)2 and CDK6 play an important role in tumor suppressor of non-small cell lung cancer [26]. We found the expression of hsa-miR-186-5p was significantly in Li population, in the development of COPD and mechanism of action it has been reported rarely, worthy of our study was to investigate in depth.

Hsa-mir-196b abnormal expression in some tumor, reminder us may be participate in certain types of cancer development [28, 29]. Lu et al found in oral cancer high expression. Liao et al found that miR-196b in gastric cancer high expression, interfere with the over expression of miR-196b can significantly induce gastric cancer cell migration and invasion [30]. Hulf et al and other studies have shown that miR-196b in epigenetic regulation levels decline in prostate cancer [31]. Ozsait et al found up-regulated expression in nasopharyngeal carcinoma [32]. Guan et al found that, the high levels of miR-196b were associated with poor prognosis in glioblastoma and anaplastic astrocytoma patients [33]. Meanwhile, our study proved hsa-miR-196b-5p was under expressed of COPD patients in Han population. These results suggested that hsa-miR-196b-5p play an important role in the occurrence of diseases.

Several important limitations to gene expression analyses must be considered in our study. For example, studies on larger numbers of COPD samples needed to validate the current findings and to address the variability in cellular composition of the tissues, given heterogeneous nature of disease. More studies are also needed to determine the functions of these significantly changed genes in the pathogenesis of COPD, and to establish whether DNA sequence variation within these genes causes to predict COPD.

In conclusion, we used RT-PCR technology to analyze the different miRNA expression between COPD in Li and Han population, and 8 miRNAs were associated with COPD. We believe that this is just the beginning of illustrations of the ways in which miRNAs are involved in COPD. MicroRNA upregulated and down-regulated can effectively adjust the biological process of these diseases; prompted MicroRNA in the respiratory system disease prevention and treatment plays an important role in the

process. It may represent a novel tool which contributes to potential cancer therapy. MicroRNA as a new drug targets and biomarkers can implement individualized medication for different treatment to the patients. These approaches not only provide molecular signatures of COPD but also can serve as biomarkers to help in the diagnosis, treatment, outcome and response to therapy for COPD patients.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81260014). The authors declare that they have no competing interests. We thank all of the participants for their involvement in this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yipeng Ding, Department of Emergency, Hainan General Hospital, #19, Xiuhua Road, Haikou 570311, Hainan, China. Tel: +86-898-66222502; Fax: +86-898-66222502; E-mail: dingyipenghainan@163.com; Dr. Tianbo Jin, School of Life Sciences, Northwest University, 386, 229 North Taibai Road, Xi'an 710069, Shaanxi, China. Tel: +86-29-88302831; Fax: +86-29-88302-831; E-mail: tianbojin1973@163.com

References

[1] Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD and Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013; 187: 347-365.

[2] Lopez AD and Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002-2030. Ann Trop Med Parasitol 2006; 100: 481-499.

[3] Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

[6] Latronico MV and Condorelli G. MicroRNAs and cardiac pathology. Nat Rev Cardiol 2009; 6: 419-429.

[7] Cuellar TL and McManus MT. MicroRNAs and endocrine biology. J Endocrinol 2005; 187: 327-332.

[8] Mehler MF and Mattick JS. Non-coding RNAs in the nervous system. J Physiol 2006; 575: 333-341.

[9] Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, Kaminski N and Abraham E. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med 2010; 207: 1589-1597.

[10] Polikepahad S, Knight JM, Naghavi AO, Oplt T, Creighton CJ, Shaw C, Benham AL, Kim J, Soibam B, Harris RA, Coarfa C, Zariff A, Milosavljevic A, Batts LM, Kheradmand F, Gunaratne PH and Corry DB. Proinflammatory role for let-7 microRNAS in experimental asthma. J Biol Chem 2010; 285: 30139-30149.

[11] Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J; Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007; 176: 532-555.

[12] Kloosterman WP and Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006; 11: 441-450.

[13] Kosaka N, Iguchi H and Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 2010; 101: 2087-2092.

[14] Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ and Wang K. The microRNA spectrum in 12 body fluids. Clin Chem 2010; 56: 1733-1741.

[15] Ferracin M, Veronese A and Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. Expert Rev Mol Diagn 2010; 10: 297-308.

[16] Sheedy FJ and O'Neill LA. Adding fuel to fire: microRNAs as a new class of mediators of inflammation. Ann Rheum Dis 2008; 67 Suppl 3: iii50-55.

[17] Papagiannakopoulos T and Kosik KS. MicroRNAs: regulators of oncogenesis and stemness. BMC Med 2008; 6: 15.

[18] Kumar MS, Lu J, Mercer KL, Golub TR and Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39: 673-677.

[19] Xie YF, Shu R, Jiang SY, Song ZC, Guo QM, Dong JC and Lin ZK. miRNA-146 negatively

regulates the production of pro-inflammatory cytokines via NF-kappaB signalling in human gingival fibroblasts. J Inflamm (Lond) 2014; 11: 38.
[20] Patnaik SK, Kannisto E, Knudsen S and Yendamuri S. Evaluation of microRNA expression profiles that may predict recurrence of localized stage I non-small cell lung cancer after surgical resection. Cancer Res 2010; 70: 36-45.
[21] Raponi M, Dossey L, Jatkoe T, Wu X, Chen G, Fan H and Beer DG. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res 2009; 69: 5776-5783.
[22] Patnaik SK, Kannisto E, Mallick R and Yendamuri S. Overexpression of the lung cancer-prognostic miR-146b microRNAs has a minimal and negative effect on the malignant phenotype of A549 lung cancer cells. PLoS One 2011; 6: e22379.
[23] Bhaumik D, Scott GK, Schokrpur S, Patil CK, Campisi J and Benz CC. Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. Oncogene 2008; 27: 5643-5647.
[24] Bostjancic E, Zidar N and Glavac D. MicroRNA microarray expression profiling in human myocardial infarction. Dis Markers 2009; 27: 255-268.
[25] Zhou L, Qi X, Potashkin JA, Abdul-Karim FW and Gorodeski GI. MicroRNAs miR-186 and miR-150 down-regulate expression of the pro-apoptotic purinergic P2X7 receptor by activation of instability sites at the 3'-untranslated region of the gene that decrease steady-state levels of the transcript. J Biol Chem 2008; 283: 28274-28286.
[26] Cai J, Wu J, Zhang H, Fang L, Huang Y, Yang Y, Zhu X, Li R and Li M. miR-186 downregulation correlates with poor survival in lung adenocarcinoma, where it interferes with cell-cycle regulation. Cancer Res 2013; 73: 756-766.
[27] Bakre A, Mitchell P, Coleman JK, Jones LP, Saavedra G, Teng M, Tompkins SM and Tripp RA. Respiratory syncytial virus modifies microRNAs regulating host genes that affect virus replication. J Gen Virol 2012; 93: 2346-2356.
[28] Lui WO, Pourmand N, Patterson BK and Fire A. Patterns of known and novel small RNAs in human cervical cancer. Cancer Res 2007; 67: 6031-6043.
[29] Schotte D, Chau JC, Sylvester G, Liu G, Chen C, van der Velden VH, Broekhuis MJ, Peters TC, Pieters R and den Boer ML. Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia 2009; 23: 313-322.
[30] Tsai KW, Hu LY, Wu CW, Li SC, Lai CH, Kao HW, Fang WL and Lin WC. Epigenetic regulation of miR-196b expression in gastric cancer. Genes Chromosomes Cancer 2010; 49: 969-980.
[31] Hulf T, Sibbritt T, Wiklund ED, Bert S, Strbenac D, Statham AL, Robinson MD and Clark SJ. Discovery pipeline for epigenetically deregulated miRNAs in cancer: integration of primary miRNA transcription. BMC Genomics 2011; 12: 54.
[32] Ozsait B, Komurcu-Bayrak E, Levula M, Erginel-Unaltuna N, Kahonen M, Rai M, Lehtimaki T and Laaksonen R. Niemann-Pick type C fibroblasts have a distinct microRNA profile related to lipid metabolism and certain cellular components. Biochem Biophys Res Commun 2010; 403: 316-321.
[33] Guan Y, Mizoguchi M, Yoshimoto K, Hata N, Shono T, Suzuki SO, Araki Y, Kuga D, Nakamizo A and Amano T. MiRNA-196 is upregulated in glioblastoma but not in anaplastic astrocytoma and has prognostic significance. Clin Cancer Res 2010; 16: 4289-4297.