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Differential Expression of MicroRNAs in Chronic Obstructive Pulmonary Disease*

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Abstract

Background. This study aimed to investigate the associations of miRNA with COPD patients.

Objectives. Chronic obstructive pulmonary disease (COPD) is characterized by progressive and largely irreversible airflow limitation. COPD is one of the most common causes of death globally and it is still a serious public health problem worldwide. Pathogenesis of COPD is multifactorial including genetics and environmental factors.

Material and Methods. Sixty patients who were diagnosed with COPD according to GOLD guidance and 40 controls were involved in the study. This study was separated into four groups according to GOLD guidance. miR_16, miR_17, miR_29c, miR_92, miR_125, miR_126, miR_146, miR_155, miR_181, mir_122 expressions from the total miRNAs obtained were worked on by using real time-PCR method. The p-values are calculated based on a Student's *t*-test of the replicate $2^{(-\Delta \text{Ct})}$ values for each gene in the control group.

Results. The miRNAs expressions in normal and COPD patients were found differentially. The miR-29c ($p = 0.043$) and -126 ($p = 0.012$) were found significantly different compared to control group. When their expressions are evaluated according to stage, miR-92 expression showed down regulation stage II and no change was observed in other miRNAs. miR-29c and miR-126 expressions showed significant differences in stage III and only miR-126 expression showed significant difference in stage IV.

Conclusions. These results show that miRNA evaluations may give information about the diagnosis, staging and prognosis of the disease. In this study, we demonstrated that miR-29c and -126 are essential for the development of COPD (Adv Clin Exp Med 2016, 25, 1, 21–26).

Key words: COPD, MicroRNAs, smoking cigarettes.

Chronic obstructive pulmonary disease (COPD) is a widespread, preventable and remediable disease, which is usually progressive, characterized by persistent air flow constraint and associated with chronic inflammatory response against harmful particles and gases in airways and pulmonary. As a result of progressive obstruction of air flow; chronic inflammation, shortness of breath, chronic cough and phlegm develop in pulmonary. It is known that genetic variants and environmental factors have a role in the pathogenesis of this disease. Smoking and/or chronic exposure to irritants takes an important place among the environmental factors [1–3].

MicroRNAs (miRNA) are 19–22 nucleotide length RNAs that do not perform protein coding and mostly regulate the expression of target mRNA in post-transcriptional level by silencing it. miRNAs play a critical role in several biological processes such as apoptosis, cell differentiation, proliferation, DNA damage repair, angiogenesis, stress response and stem cell division [4]. Therefore, the changes in miRNA expressions are associated with the development and progression of human diseases like cancer, cardiovascular diseases, inflammation and asthma [5]. miRNA's role in inflammatory pulmonary diseases becomes more important increasingly.

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Many studies make us think that miRNAs act in pulmonary diseases including airway diseases like COPD [6, 7]. However, there are very few studies that reveal the relation between COPD and miRNAs. Definition of miRNA expression patterns in patients with COPD may help readability of the disease mechanism.

Research on this subject has increased gradually in recent years. The aim of our study is to work on the miRNA expression for patients with COPD and contributing to shedding light on the pathogenesis of the disease.

Material and Methods

This study was carried out by Firat University Medical Faculty, Department of Chest Diseases and Department of Medical Genetics. The study was approved by the Firat University Medical Faculty ethics committee (04.19.2012 and no. 119) and informed consent forms were received from the cases. Sixty cases were involved in the study by taking their symptoms, physical examination, laboratory findings and pulmonary function test parameters into consideration according to COPD diagnosis GOLD guidance [8]. Ages, gender, medical and smoking histories of cases were questioned. Existence of systemic, allergic, neoplastic or immunologic disease, diagnosis of reversibility in respiratory functions, bullous emphysema related to α 1-antitrypsin deficiency or the existence of accompanying other pulmonary disease were determined as the criteria of exclusion from the study. Besides patients with COPD, 40 cases, which do not have any pulmonary or systemic diseases, have normal pulmonary function with regards to spirometry, do not have any histories of infection or regular drug use within the last 1 month and do not smoke cigarettes, were chosen randomly and determined as a voluntary control group. Patients with COPD were classified as stage III according to GOLD classification. Due to the fact that stage I had only 1 patient, it was combined with stage II and came up as a single stage (19 patients); 22 patients were evaluated in stage III and 19 patients were evaluated in stage IV. The number of smokers was 37 and non-smokers 23.

For the study, 2 cc of bloods were taken from patient and control groups to tubes containing EDTA. Serum was obtained from the blood and a total miRNAs were received from serums by using genomic miRNeasy Mini extraction Kit (QIAGEN Sample & Assay Technologies, Germany). miR_16, miR_17, miR_29c, miR_92, miR_125, miR_126, miR_146, miR_155, miR_181, miR_122 expressions from the total miRNAs obtained were

Table 1. The accession numbers of miRNA primers

miRNA ID	miRNA accession number
hsa-miR-16	MIMAT0000069
hsa-miR-17	MIMAT0000070
miR29c	MIMAT0000681
miR92	MIMAT0000222
miR125b	MIMAT0000423
miR126	MIMAT0000444
miR146	MIMAT0000449
miR155	MIMAT0004658
miR181a	MIMAT0000256
miR221	MIMAT0000278

worked on by using real time-PCR (RT-PCR) method (Rotor Gene 6000 Real-Time PCR Machine). The access numbers of primers were given in Table 1.

Respiratory function test was performed on the entire cases in spirometry laboratory through Superspiro (Micromedical Limited, Rochester, England) device and FEV1, FVC, FEV1/FVC values were measured. The measurement was performed under room temperature by closing the noses with nippers at least three times during the recovery period and the best values were obtained.

Statistical Tests

SPSS 12.0 computer program was used in the evaluation of the data. Results were presented as mean $\pm$ standard deviation. $P < 0.05$ values were accepted as statistically significant. Variance analysis was used for evaluating whether there is a difference between the data obtained from the groups. The p-values are calculated based on a Student's *t*-test of the replicate $2^{-\Delta\Delta Ct}$ values for each gene in the control group.

Results

Sixty patients (17 female, 43 male), who were diagnosed COPD according to GOLD guidance and 40 controls (13 female, 27 male) were involved in the study. General features of the patients were summarized in Table 2. Differentially expressed miRNAs in normal and COPD patients showed in Table 3. The miR-126 ($p = 0.012$) and miR-29c ($p = 0.043$) were found significantly different compared to control group (Table 3).

Fold-changes in the patient group compared to the control group are shown in Fig. 1. When

Table 2. General features of the patients

	Patient (n : 60)	Control (n : 40)
Age	50–89	46–70
Male	43 (71.7%)	27 (67.5%)
Female	17 (28.3%)	13 (32.5%)
Ph	7.40 ± 0.05	
pCO ₂	49.02 ± 12.30	
HCO ₃	28.30 ± 5.60	
FVC	46.65 ± 16.67	
FEV1/FVC	64.30 ± 13.70	

the control group was compared to stage II, III, and IV, only mir-92 expression was found to be down-regulated in stage II, although the difference did not reach statistical significance ($p > 0.05$). In stage III, only mir-29c and mir-126 expressions were up-regulated, and the difference was found to be significant compared to the control group ($p < 0.005$). In stage IV, only mir-126 expression showed significant difference ($p < 0.005$) (Table 4).

Table 5 shows changes in miRNA expression in non-smokers compared to smokers as the control group. Although miR-17 and -29c expressions were up regulated, only the difference in miR-146 expression reached statistical significance ($p < 0.005$).

Table 3. Differentially expressed miRNAs in normal and COPD patients

miRNA	Control		Case	
	2 [^] (-Avg. (Delta(Ct)))	2 [^] (-Avg. (Delta(Ct)))	fold change	p-value
miR16	0.865	0.939	1.086	0.569
miR17	1.877	1.332	0.709	0.220
miR29c	1.359	2.100	1.545	0.043
miR92	61.229	48.618	0.794	0.175
miR125	1.159	1.348	1.163	0.353
miR126	2.433	5.022	2.063 [^]	0.012
miR146	0.865	0.939	1.086	0.569
miR155	0.865	0.963	1.113	0.310
miR181	1.143	1.353	1.183	0.688
miR221	1.094	0.943	0.862	0.293

[^], up-regulation.

Table 4. The expression profiles of selected miRNAs in different staged COPD (according to GOLD guidance)

miRNA	Stages I and II		Stage III		Stage IV	
	fold change	p-value*	fold change	p-value*	fold change	p-value*
miR16	1.155	0.301	0.923	0.617	1.155	0.377
miR17	0.605	0.165	0.837	0.828	0.689	0.299
miR29c	1.066	0.333	2.093 [^]	0.009	1.467	0.070
miR92	0.399 [#]	0.573	0.903	0.502	1.034	0.157
miR125	1.051	0.660	1.200	0.542	1.138	0.560
miR126	1.356	0.063	2.152 [^]	0.009	1.895	0.020
miR146	1.155	0.301	0.923	0.617	1.155	0.377
miR155	1.156	0.302	1.011	0.484	1.156	0.378
miR181	1.030	0.818	1.266	0.786	1.186	0.646
miR221	0.921	0.469	0.730	0.553	0.914	0.532

* p-value (comparing to control group); [^], up-regulation; [#], down-regulation.

Fig. 1. Fold-changes in the patient group compared to the control group

Table 5. The expression profiles of selected miRNAs in smokers or non-smokers

miRNA	With smokers (n:37) (control group)		With nonsmokers (n:23)	p-value
	2 [^] (-Avg. (Delta(Ct)))	2 [^] (-Avg. (Delta(Ct)))	fold change	
miR16	1.006	0.797	0.792	0,079
miR17	1.461	3.258	2.229 [^]	0.094
miR29c	1.993	4.803	2.409 [^]	0.400
miR92	232.020	282.977	1.219	0.500
miR125	1.791	2.622	1.464	0.286
miR126	13.876	23.729	1.710	0.177
miR146	0.969	0.729	0.753	0.016
miR155	0.997	0.964	0.966	0.414
miR181	1.642	2.270	1.382	0.864
miR221	1.036	0.943	0.871	0.594

*p-value; [^], up-regulation.

Discussion

COPD is a heterogeneous disease associated with the abnormal inflammatory response given by pulmonary against harmful particles and gases. It was shown that an increase of COPD for smokers caused an increase in inflammatory response and changes in gene expression in respiration epithelium, in parallel with the development of structural abnormalities both in airways and pulmonary

parenchyma [7, 9]. Observations of these changes in pulmonary showed an increase in miRNA expression studies recently. Due to its being a disease at a price, COPD impose an important burden on the health systems in many countries. Therefore, revealing the mechanisms, which take part in COPD pathogenesis, rises in importance.

MiRNAs act as molecular keys that fine-tune the signals for specific cellular events in the development of various organs including pulmonary.

Deregulation in miRNA expression is associated with human diseases including cancer and inflammatory [10]. Several miRNAs such as miR-16, miR-26a, miR-29, miR-17-92 cluster, miR-146 and miR-155 are thought to be playing role in pulmonary development [11]. miR-155 was defined as a component of primary response of several inflammatory mediators in various cell types and it was reported that it has a role in the differentiation of T cells as Th1 and Th2 [12]. miR-21 shows abnormal expression for vascular diseases in pulmonary [13]. It was revealed that miRNA-26 behaved as a regulatory factor in smooth muscle hypertrophy (enlargement) and let-7 miRNA increased the inflammatory related to asthma [14]. It is believed that miR-17-92 cluster (take part in 13q31.3) expressions of pulmonary to be high during embryonic development and low in adulthood, has a role in the regulation of pulmonary development [11, 15].

Qin et al. stated that miRNA-221 expression exposed to up-regulation in patients with asthma and that it could be a functional regulator for asthma pathogenesis [6]. Chen et al. stated that miR-146a expression was down-regulated in the inflammatory process of pulmonary diseases, and Bockmeyer et al. stated that there was an increase in miR-143/145 expression [12, 16]. It was asserted that a decrease in miR-146a could be one of the determiners of inflammatory state and COPD progress. Ezzie et al. asserted that an increase in miR-15/107 could have role in COPD pathogenesis [17]. Recent studies support the hypothesis of miR-21, miR-146a and miR-146b have role in the regulation of inflammatory in pulmonary [11]. In the miRNA expression profile work carried out according to the controls both on rats and humans

that were exposed to ambient cigarette smoke, it was stated that miR-26, -30, -122, -123, -125 and -146 were exposed to down-regulation [18].

In the present study, miR-17 and -29c expressions were up-regulated in smokers and non-smokers; however, only the changes in miR-146 expression reached statistical significance (Table 5). This finding suggests that smoking causes changes in gene expression in respiratory epithelium considering the fact that smoking is a basic risk factor for the development of COPD. When the changes in gene expression were evaluated according to the stages, mir-92 expression showed down-regulation stage II and no change was observed in other miRNAs. Mir-29c and mir-126 expressions showed significant differences in stage III and only mir-126 expression showed significant difference in stage IV ($p < 0.005$) (Table 4). These findings suggest that the evaluation of miRNA expressions could provide information about the diagnosis, disease staging, and prognosis.

Studies on pulmonary diseases in recent years have also revealed that up-regulated and down-regulated expressions of several miRNAs have an active role in the pathogenesis of pulmonary diseases. In this study, we aimed at giving information about the miRNA expression alterations as a biomarker applicant with regard to disease development, prognosis and therapeutic intervention.

miRNAs such as miR-125 and -221 have the potential to become novel biomarkers for predicting prognosis and therapeutic efficacy in COPD. For more information about the role of miRNAs in physiological and pathological cases in pulmonary compartment, forward-looking studies are needed both for animal models and for different ethnic groups according to stage.

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