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MICRORNA PROFILING IN POTENTIAL LUNG RECIPIENTS

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MicroRNAs are small RNA molecules stable in blood serum (plasma) samples. Their level of expression is associated with the severity and nature of physiological and pathological processes in the body. **Aim:** to evaluate the expression levels of five microRNAs (miR-27, miR-101, miR-142, miR-339 and miR-424) in potential lung recipients with end-stage chronic lung diseases of various etiologies. **Materials and methods.** The study included 16 patients with end-stage chronic lung diseases (potential lung recipients) aged 4 to 74 years (average 36 ± 18). Among them were two children (12.5%) – girls aged 4 and 14 years, and 14 adults aged from 21 to 74 (40 ± 16) years – 6 men (42.9%) and 8 women. The control group consisted of 12 healthy individuals. The main diseases that caused severe respiratory failure were: cystic fibrosis (n = 5), primary pulmonary hypertension (PPH; n = 4), pulmonary fibrosis of various etiologies (idiopathic pulmonary fibrosis – 1; pulmonary fibrosis associated with exogenous allergic alveolitis – 1; radiation-induced pulmonary fibrosis – 1), lymphangioleiomyomatosis (n = 2), histiocytosis (n = 1) and pulmonary emphysema (n = 1). MicroRNA expression was detected through real-time PCR. The level of microRNA expression in plasma was estimated in accordance with instructions for reagent kits (Qiagen, USA). **Results.** The levels of miR-27, miR-101 and miR-339 in potential lung recipients were significantly higher than in healthy individuals. The levels differed depending on the etiology of diseases: the levels of miR-27, miR-101, miR-142 and miR-339 were higher in patients with cystic fibrosis than in healthy individuals; in patients with other lung diseases, only miR-101 levels where higher than in healthy individuals. The miR-424 level in healthy individuals did not differ from that in potential lung recipients or in subgroups. **Conclusion.** Results obtained show the features of a number of microRNA levels (miR-27, miR-101, miR-142, and miR-339) under certain lung diseases and suggest a possibility of a diagnostic value in patients with chronic respiratory failure during pre-transplant examination.

Keywords: microRNA, biomarker, miR-27, miR-101, miR-142, miR-339, miR-424, cystic fibrosis, chronic respiratory failure.

MicroRNA is a wide range of small non-coding RNA molecules, stable in blood serum (plasma), performing regulatory functions, with the level of expression associated with the severity and nature of physiological and pathological processes in the body [1–3]. In this context, it is assumed that data on changes in the expression of certain microRNA types in recipients of solid organs may be helpful for early preclinical diagnosis of post-transplant complications [4, 5]. The change in the expression of certain types of microRNAs at the rejection of transplanted solid organs was found [6, 7].

The lung transplantation (LT) is an effective treatment for such chronic terminal lung diseases as primary pulmonary arterial hypertension (PAH), cystic fibrosis, chronic obstructive pulmonary disease, and others.

Despite significant advances in surgical technology and improvement of the immunosuppressive therapy which increased survival and improved the quality of life for recipients of solid organs, the vital task is to search for and validate biomarkers that are potentially suitable for non-invasive or minimally invasive diagnosis of post-transplant complications and prediction of long-term clinical outcomes [8–9].

Purpose of the study. Evaluation of microRNA expression level (miR-27, miR-101, miR-142, miR-339, and miR-424) in patients suffering from chronic lung diseases of different aetiology in the terminal stage (potential lung recipients).

MATERIALS AND METHODS

The study included 16 patients with terminal-stage lung diseases aged 4 to 74 (36 ± 18), among them two children (12.5%), girls aged 4 and 14 years, and 14 adult patients aged 21 to 74 (40 ± 16) years, 6 (42.9%) men and 8 women. The main diseases to cause the severe respiratory failure were cystic fibrosis (n = 5), pulmonary arterial hypertension (PAH; n = 4), pulmonary fibrosis of various etiologies (idiopathic pulmonary fibrosis – 1;

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pulmonary fibrosis as the outcome of exogenous allergic alveolitis – 1; post-radiation pulmonary fibrosis – 1), lymphangioleiomyomatosis (n = 2), histiocytosis (n = 1), and pulmonary emphysema (n = 1). The control group consisted of 12 healthy individuals. The average age and ratio of men and women in the control group did not differ from those in the test group.

Isolation of microRNA from the plasma of peripheral blood

The peripheral blood samples of patients were collected in disposable tubes with an anticoagulant ethylene diamine acetic acid (EDTA), centrifuged for 10 minutes at 3,000 rpm. Blood plasma was separated from the cell pellet and immediately frozen at –20 °C. RNA was isolated from 100 µl of blood plasma using SerumPlasma kits (Qiagen, USA) with the preliminary addition of 1.6 x 108 copies of cel-miR-39 synthetic microRNA (Qiagen) after plasma incubation with Qiazol phenolic mixture. Cel-miR-39 was used as an internal control of the efficiency of RNA isolation, synthesis of complementary DNA (cDNA), and quantitative polymerase chain reaction (PCR) in real time.

Real-time reverse transcription and quantitative PCR

MicroRNAs from each sample were converted into cDNA in a reaction mixture (20 µl) containing 1xmiScriptHiSpecBuffer buffer, 1xmiScriptNucleicsMix nucleotide mixture at t = 37 °C for 60 minutes, followed by incubation at 95 °C for 5 minutes, cooling on ice and dilution of sample volume with deionized water up to 200 µl. The synthesized cDNA (2 µl) served as the matrices in real-time PCR, using primers specific for the studied microRNAs: miR-27, miR-101, miR-142, miR-339, miR-424, cel-miR-39 (miScriptPrimerassay, Ce_miR-39_1, Qiagen), and the miScriptSYBRGreenPCRKit kit (Qiagen). PCR reaction conditions: 15 minutes at t = 95 °C followed by 40 cycles of 15 seconds at t = 94 °C, 30 seconds at t = 55 °C, and 30 seconds at t = 70 °C in the CFX 96 amplifier (Biorad). The intensity of microRNA expression was shown in relative units equivalent to $2^{-\Delta Ct}$, where ΔCt is the operating values of changes in the product obtaining cycle relative to the internal control of microRNA cel-miR-39 expression.

Statistical data processing

Statistical analysis of the results was carried out by Statistica v.13.0, StatSoftInc (USA software package). Spearman correlations and the Mann–Whitney U-test were used to compare the independent variables. The critical level of significance was taken 5% and the null hypothesis was rejected at $p < 0.05$. The obtained expression indices were checked for normal values distribution.

RESULTS AND DISCUSSION

In patients with severe respiratory failure that developed as the outcome of chronic lung diseases, microRNA expression rates varied over a wide range. The distribution of values differed from normal; therefore, in the present study, the results are provided by the values of the median and interquartile range expressed in relative units (RU). Table 1 shows the results of a comparative analysis of the expression levels of the five studied types of microRNAs (miR-27, miR-101, miR-142, miR-339, and miR-424) in men and women suffering from lung diseases, and the significance of differences between them is indicated (p).

Table 1

Comparative analysis of microRNA plasma expression levels in male and female patients

MicroRNA	Sex		Statistical significance (p)
	Male (n = 6)	Female (n = 10)	
miR-27	0.071 [0.059; 0.33]	0.030 [0.016; 0.067]	0.09
miR-101	0.057 [0.031; 0.083]	0.046 [0.022; 0.057]	0.63
miR-142	0.011 [0.007; 0.072]	0.006 [0.003; 0.011]	0.31
miR-339	0.023 [0.001; 0.130]	0.004 [0.001; 0.076]	0.43
miR-424	0.007 [0.005; 0.014]	0.002 [0.001; 0.008]	0.12

Note. Data is provided as: median [interquartile range].

An analysis of the expression levels of the studied microRNAs in potential lung recipients showed no gender differences.

Table 2 shows the results of an analysis of the relationship between microRNA expression levels and the age of patients waiting for lung transplantation.

Table 2

Correlation analysis of microRNA expression levels and the age of the patients

MicroRNA	Correlation coefficient (r)	Statistical significance (p)
miR-27	–0.50	0.04
miR-101	–0.48	0.06
miR-142	–0.41	0.12
miR-339	–0.33	0.22
miR-424	–0.06	0.83

A significant negative correlation was found between the miR-27 expression level and the age of patients (r = –0,50, p = 0,04); for other types of microRNAs, no age correlation was found.

Fig. 1 shows the results of a comparative analysis of the expression of the studied microRNAs in healthy in-

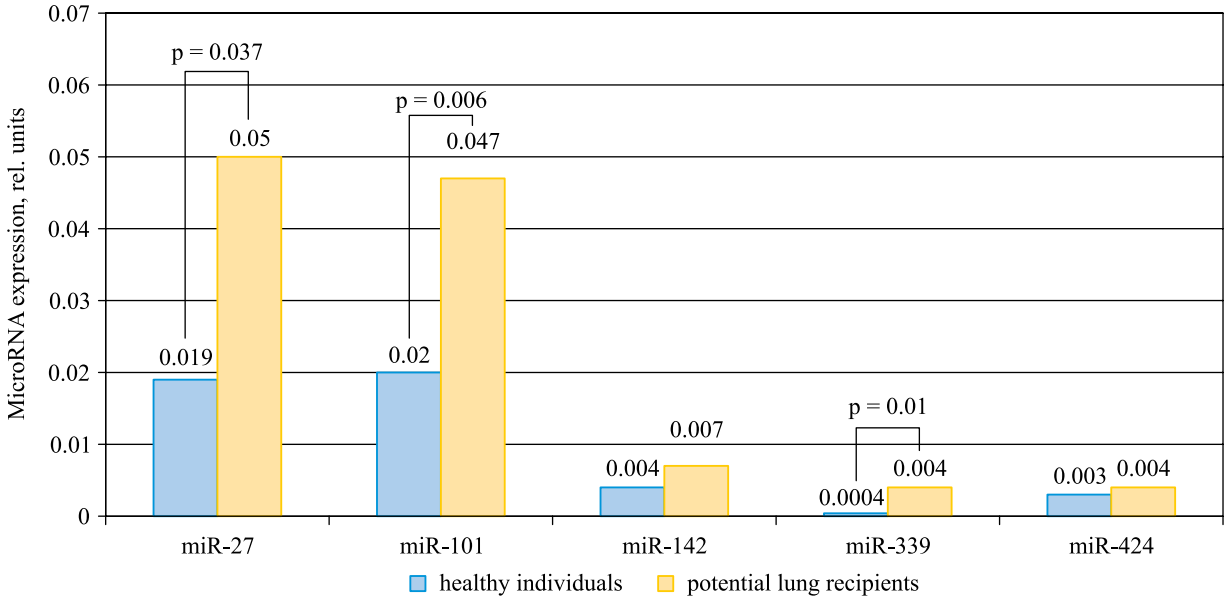

Fig. 1. Comparative analysis of microRNA levels (miR-27, miR-101, miR-142, miR-339 and miR-424) in healthy individuals and potential lung recipients

dividuals and patients suffering from respiratory failure, potential lung recipients.

In patients waiting for lung transplantation, the expression levels of three of five types of studied microRNAs were significantly higher than in healthy individuals: miR-27 ($p = 0.037$), miR-101 ($p = 0.006$), and miR-339 ($p = 0.01$)

The differences in microRNA expression were found to be associated with aetiology of the disease that caused the development of respiratory failure. In five patients, the cause of respiratory failure was an infectious mediated disease – cystic fibrosis (31.3% of the total number of patients). The remaining 11 patients suffered from the diseases with obstructive, restrictive processes as the main pathogenetic factors; vascular disorders (PAH, pulmonary fibrosis of various aetiologies, emphysema, etc.)

The analysis showed that miR-101 expression was significantly higher in patients with cystic fibrosis ($p = 0.01$) and those suffering from other lung diseases ($p = 0.03$, Fig. 2, a) compared with the group of healthy individuals.

The expression level of miR-27 in patients with cystic fibrosis was higher in comparison with both healthy individuals ($p = 0.001$, Fig. 2, b) and other patients ($p = 0.01$). It should be noted that the differences were not caused by the younger age of patients with cystic fibrosis ($p = 0.07$).

A higher level of miR-142 and miR-339 expression in comparison with healthy individuals was detected only in patients with cystic fibrosis ($p = 0.04$, Fig. 2, c; $p = 0.01$, Fig. 2, d, respectively).

The expression level of miR-424 did not differ from that in healthy individuals, neither in the group of potential recipients nor in subgroups of patients with different aetiologies of the disease (Fig. 2, e).

The important regulatory role of microRNAs in the development of pathological processes is described in many studies of recent years, which stimulates the active study of small molecules from the perspective of their potential significance as biomarkers of posttransplantational complications and use as potential targeted therapy for rejection of solid organs in recipients [10–12].

To date, a sufficiently large number of different microRNA types have been identified and described. Five of them were selected for this study, presumably playing a role in the development of diseases of the lungs and cardiovascular system and potentially significant for the diagnosis of posttransplantational complications in heart and lung recipients [10, 14].

Significant differences in the expression of miR-101, miR-142 were described in patients with heart and lung diseases [13, 14]. In patients with idiopathic pulmonary fibrosis, miR-101 has been shown to inhibit the proliferation and activation of fibroblasts, being a potential therapeutic target [15]. Data has been published on the possible involvement of miR-142 in the regulation of haematopoiesis. Besides, this type of microRNA is expressed in many tissues and performs important functions in inflammatory, immune, infectious, oncological, and fibrotic processes after transplantation of donor organs [16].

CONCLUSION

The results of this study showed that the expression profile of various types of microRNAs in patients with chronic respiratory failure waiting for lung transplantation differs from that in healthy individuals. Moreover,

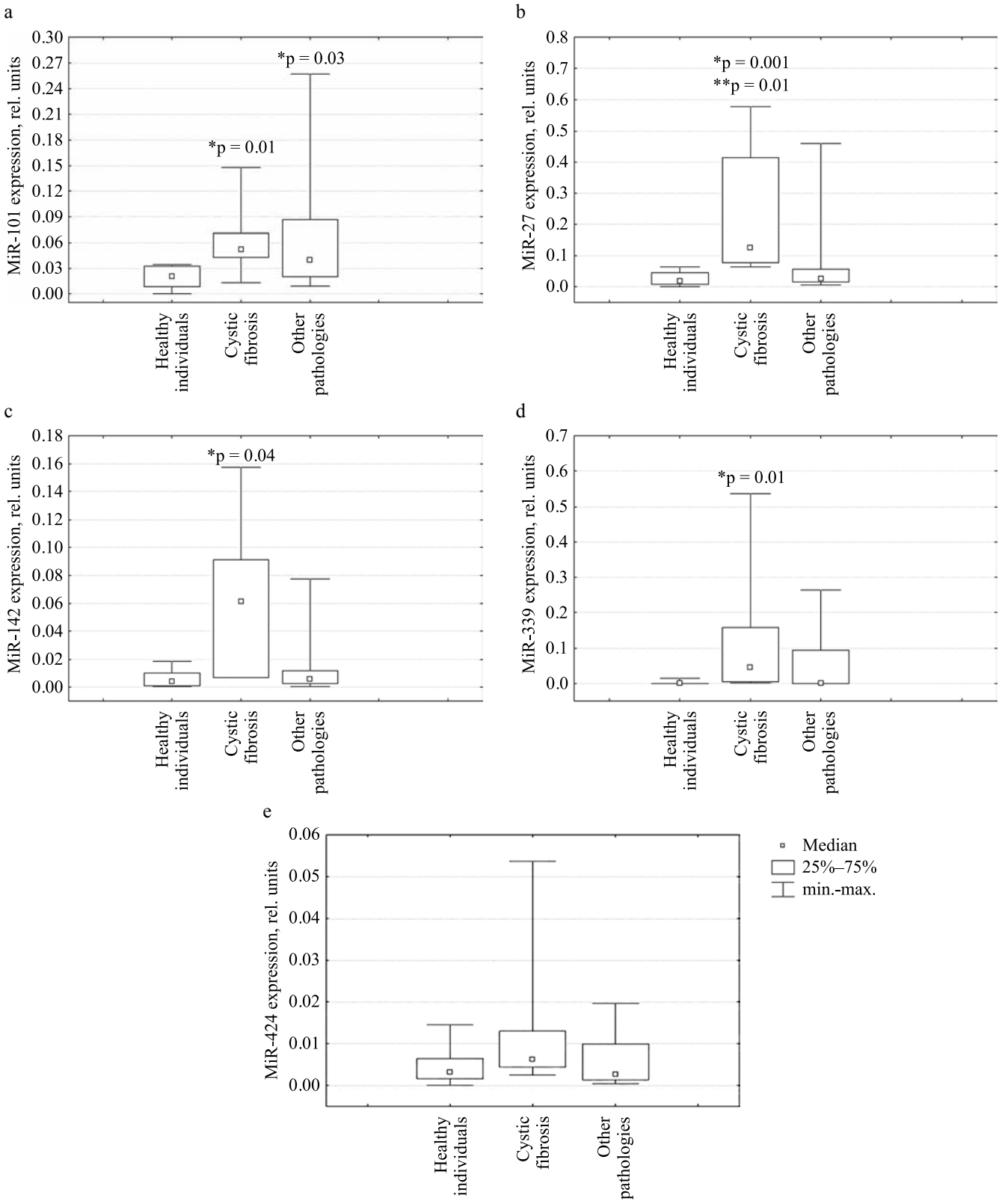

Fig. 2. Comparative analysis of miR-101 (a), miR-27 (b), miR-142 (c), miR-339 (d) and miR-424 (e) levels in healthy individuals, patients with cystic fibrosis and other lung pathologies. * – compared to healthy individuals; ** – compared to patients with other lung pathologies

the expression of certain types of microRNAs is associated with the aetiology of lung disease: in patients with cystic fibrosis, expression levels of miR-27, miR-101, miR-142, and miR-339 were significantly higher in comparison both with healthy individuals and patients with less pronounced infection mediated disorders.

The literature data, as well as the results of this study, indicate the relevance of studying the levels of microRNA expression in patients with severe pulmonary pathology, as well as in lung recipients. To clarify the possible clinical significance of the application of the expression levels of these molecules, further study of their biological functions and diagnostic efficacy in this group of patients is required.

The authors declare no conflict of interest.

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