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Expression of microRNA-590 in patients with chronic coronary heart disease, atrial fibrillation, and their combination

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Abstract

Background. The role of microRNAs in the processes of remodeling, proliferation, and fibrogenesis of myocardial cells is not clear enough.

Aim. Analysis of the microRNA-590 expression level in patients with chronic coronary heart disease, atrial fibrillation, as well as their combination to assess the potential prognostic significance of the expression level.

Material and methods. The study included 94 patients divided into three clinical groups: the first — with non-valvular atrial fibrillation without coronary heart disease (39 people); the second — with non-valvular atrial fibrillation and coronary heart disease (22 patients); the third — with ischemic heart disease without atrial fibrillation (23 patients). The comparison group consisted of 10 people without atrial fibrillation and coronary heart disease. Venous blood was taken from all subjects, from the plasma of which microRNA was isolated. The relative level of microRNA expression was estimated based on real-time polymerase chain reaction data obtained during the reaction on a cycler using commercial TaqMan probes and primers. The statistical significance of differences between groups was determined using one-way analysis of variance, followed by post-hoc analysis using Tukey's contrasts, differences were considered statistically significant at $p < 0.05$. The Shapiro–Wilk test was used to assess the normality of the distribution of residuals.

Results. A statistically significant decrease in the expression level of microRNA-590 was registered in the third ($p=0.0104$) and in the second ($p=0.0046$) groups compared with the control group, as well as in patients with atrial fibrillation and left atrial dilatation ($p=0.0313$), with recurrent arrhythmias after radiofrequency ablation ($p=0.0083$) and with permanent atrial fibrillation ($p=0.0242$).

Conclusion. Ischemic heart disease, including when combined with atrial fibrillation, and aggravating factors, such as left atrial dilatation, permanent atrial fibrillation, recurrence of arrhythmia after radiofrequency ablation, lead to a decrease in the expression level of microRNA-590.

Keywords: microRNA-590 expression, atrial fibrillation, ischemic heart disease, left atrial dilatation, permanent AF, radiofrequency ablation.

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Background

Cardiomyocytes lose the ability to proliferate in the first few months of postnatal ontogenesis. However, the hemodynamic load on the heart increases during the postnatal period, which cannot be achieved by cardiomyocyte hyperplasia. Therefore, the mechanism of myocardial hypertrophy is activated, and the size of cardiomyocytes increases through sarcomerogenesis [1].

Myocardial recovery following myocardial infarction is often limited and typically occurs through replacement of connective tissue and changes in the structural and functional properties of cardiomyocytes [2]. Due to the limited regenerative capacity of adult cardiomyocytes, it is imperative to understand the epigenetic mechanisms of normal heart development for prevention and treatment of heart failure.

K. Takahashi et al. [3] demonstrated that the ectopic expression of the transcription factors Oct4, Sox2, Klf4, and c-Myc (OSKM cocktail) can reprogram differentiated cells to pluripotency.

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Reprogramming is a gradual process that involves changes in the epigenetic landscape [4]. Revealing cell identity by understanding how reprogramming factors change the cell epigenome is an important goal in regenerative medicine [5].

Gilsbach et al. [6] demonstrated that different epigenetic programs regulate the development of cardiac myocytes and the contractile function of the human heart *in vivo*. This process is primarily controlled by embryonic genes, which may undergo decreased expression under pathological conditions. MicroRNA molecules are noncoding single-stranded ribonucleic acids (RNAs) that are 19–24 nucleotides in length, with the primary function of inhibiting the expression of protein-coding genes at the post-transcriptional level. They can serve as markers that reflect this process [1]. Circulating microRNA molecules demonstrate high specificity and sensitivity. They are stable in biological fluids and are frequently detected in blood at all stages of a disease, making them useful as biomarkers for the early diagnosis and prognosis of different pathological conditions [7].

A healthy heart contains several cardiac-specific microRNAs, including microRNA-1, microRNA-133, microRNA-208, microRNA-499, microRNA-138, and microRNA-208, which are crucial for maintaining cardiac muscle identity and function. Cardiac tissue injury leads to fibroblast differentiation and reduced myocardial contractility because of the loss of functioning myocytes. Delivery of cardiac microRNAs to reprogram fibroblasts into functioning cardiomyocyte-like cells increases cardiac contractility [5].

MicroRNA-590 [2] is of interest in investigations on the proliferative properties of cardiomyocytes, although its exact role in these processes remains debatable.

A.S. Eulalio et al. [2] demonstrated the ability of microRNA-590 and microRNA-199a to reactivate the cell cycle of adult cardiomyocytes in vivo and promote proliferation, regardless of the animal's age. Administering exogenous microRNA-590 to mice with myocardial infarction significantly reduced the damage size and almost completely recovered the heart function. Their study shows promise for treatment of cardiac pathologies caused by cardiomyocyte loss.

The objective of this study was to evaluate the potential prognostic significance of the relative expression level of microRNA-590 in patients with chronic coronary heart disease (CHD), nonvalvular atrial fibrillation (AF), and AF + CHD.

Materials and methods

Approval was obtained from the local ethical committee of Kazan State Medical University, as documented in the minutes of meeting No. 2 on February 26, 2019.

The study included 94 patients aged 40–80 years (mean age 67.4 ± 10.8 years) who underwent inpatient examination and treatment at the cardiology department of the medical unit of Kazan Federal University. Patient recruitment was conducted from October 2018 to June 2019. Sample size calculation was not evaluated because this study is pilot in nature. The study was selective and one-stage, with all patients who met the inclusion criteria being included in the study groups.

Inclusion criteria:

– Presence of nonvalvular AF (associated with arterial hypertension and diabetes mellitus) and various types and forms of uncomplicated CHD, including after radiofrequency ablation (RFA) during anamnesis;

– Presence of verified chronic CHD with or without AF.

Exclusion criteria:

– Valvular AF

– Chronic rheumatic heart disease

– Inflammatory diseases of the myocardium, endocardium, and pericardium

– Other infectious and inflammatory diseases

– Congenital and acquired heart defects with regurgitation and/or grade 2 or more stenosis

– Conditions after valve replacement

– Pacemaker implantation

– Conduction abnormalities

– Acute coronary syndrome

– Postinfarction cardiosclerosis

– Night apnea syndrome

– Thyroid dysfunction

– Stroke and/or transient ischemic attack in anamnesis

– Pulmonary artery thromboembolism in anamnesis

– Oncologic diseases

– Chronic obstructive pulmonary disease in the stage of exacerbation

– Chronic kidney disease

– Mental disorders

Informed consents were obtained from the study subjects, and all received baseline therapy, including anticoagulants, antiplatelets, antiarrhythmics, antianginals, and hypotensive drugs, in accordance with both Russian and international guidelines.

To verify or exclude CHD, all patients underwent coronary angiography or multispiral computed tomography of the coronary arteries if they were in sinus rhythm. In addition, all patients underwent echocardiography and daily Holter monitoring of electrocardiography data.

Table 1. Gender and age characteristics of patients in the two groups

Characteristics	Group 1		Group 2		Group 3		p
	n	%	n	%	n	%	
Mean age, years	62,5±12,2		70,0±6,4		62,7±10,5		0,0136
Men	16	41	10	45	15	65	0,0651
Women	23	59	12	55	8	35	
<60 years of age	15	38	0	0	8	35	—
61–70 years of age	20	51	6	27	9	39	—
71–80 years of age	4	10	16	73	6	26	—

Note: p is the Kruskal–Wallis test score.

Table 2. Clinical characteristics of patients in the two groups

Characteristics	Group 1		Group 2		Group 3		p
	n	%	n	%	n	%	
Stenosis of more than one coronary artery	—	—	18	82	23	100	0,0491
Coronary stenting	—	—	4	18	23	100	<0,0001
Left atrial dilatation	24	62	19	83	12	50	0,0487
RFA	21	54	0	0	—	—	<0,0001
AF recurrence after RFA	8	21	0	0	—	—	<0,0001
Tachysystolic AF	20	51	10	45	—	—	0,3836
Normosystolic AF	19	49	12	55	—	—	0,7719
Paroxysmal AF	22	56	12	54	—	—	>0,99
Persistent AF	13	34	5	23	—	—	0,5414
Permanent AF	4	10	5	23	—	—	0,2279
Type 2 diabetes mellitus	2	5	4	18	0	0	0,0472
Hypertension	25	64	18	81	20	87	0,1121
CHA2DS2VASc < 2	13	33	—	—	3	9	0,1318
CHA2DS2VASc >2	26	67	—	—	20	91	

Note: p, Fisher's exact test; RFA, radiofrequency ablation; CHA2DS2VASc, Risk Assessment Scale for Stroke and Thromboembolic Complications in Atrial Fibrillation (AF).

The patients were divided into three by diagnosis: group 1 included 39 individuals diagnosed with AF without signs of CHD, group 2 included 22 individuals diagnosed with chronic CHD and AF, and group 3 included 23 patients diagnosed with CHD without AF. The comparison group included 10 individuals, both men and women aged 40–80 years, without AF or signs of CHD. Tables 1 and 2 summarize the clinical and demographic characteristics of the patients in each group.

MicroRNA isolation was performed using a commercial reagent kit from SibDNA LLC (Novosibirsk). Complementary DNA was obtained using a commercial TaqMan MicroRNA Reverse Transcription Kit (ThermoFisher Scientific, USA). The relative level of microRNA expression was estimated on the basis of real-time polymerase chain reaction data obtained during the reaction on a CFX96 amplifier (BIO-RAD, USA) using commercial TaqMan probes and primers (Applied Biosystems, USA).

The expression level of microRNA-590 was calculated using the $2^{-\Delta\Delta CT}$ Relative Quantity (RQ) method, as described by Kenneth J. Livak and Thomas D. Schmittgen. This method reflects the change in the expression of microRNAs in the studied groups compared with the control group with respect to SNORD48 as reference [8]. Moreover, ΔCT is the number of cycles required to reach amplification, which is the threshold level of expression in the studied groups and RQ is a quantitative index that reflects the true activity of a given microRNA. RQ and ΔCT are inversely related to each other.

Table 3. Results of the relative expression of microRNA-590

Comparison	ΔΔCT	FC [95% CI]	Number of times the expression decreases compared to the control group	p
CHD/Control	4,41	0,047 [0,571–0,004]	21,23 [1,75–257,47]	0,0104
AF/Control	2,96	0,129 [1,098–0,015]	7,78 [0,91–66,39]	0,0658
CHD + AF/Control	4,56	0,042 [0,457–0,004]	23,65 [2,19–255,55]	0,0046
AF/CHD	–1,45	2,731 [18,288–0,408]	0,366 [0,055–2,452]	0,5061
CHD + AF/CHD	0,16	0,898 [7,814–0,103]	1,114 [0,128–9,693]	0,9992
CHD + AF/AF	1,60	0,329 [1,888–0,057]	3,042 [0,530–17,462]	0,3415

Note: FC, ratio of expression levels in comparison groups; CI, confidence interval; CHD, coronary heart disease; AF, atrial fibrillation.

The Kruskal–Wallis test was used to compare quantitative variables, followed by Dunn's test with Holm's correction as a post-hoc method. Fisher's exact test was used to compare the groups based on qualitative indicators.

Statistical significance of differences between groups was determined using one-way analysis of variance followed by post-hoc analysis using Tukey's contrasts. Statistical significance was set at $p < 0.05$. The normality of the distribution of residuals was assessed using the Shapiro–Wilk test. Binomial regression models were used to assess the association of microRNA-590 concentration with qualitative outcomes, with gender and age of patients used as correcting covariates.

Results

Patients in group 2 had higher average age than those in groups 1 and 3 ($p = 0.0261$). There were no statistically significant differences between groups 1 and 3 ($p = 0.6637$) in terms of age. Gender composition did not significantly differ between the groups.

The normality of the residuals was checked using the Shapiro–Wilk test, and the results showed no deviation from normality ($p = 0.7365$).

Statistically significant differences were found between the patient groups in terms of microRNA-590 expression ($p = 0.0043$). The group of comorbid AF patients with CHD showed the most pronounced decrease in expression level, with a 23.6-fold decrease [95% confidence interval (CI) 2.2–255.5] compared with the control group ($p = 0.0046$; Table 3). In patients with CHD but without AF, there was a 21.2-fold decrease in the expression of microRNA-590 (95% CI 1.8–257.5, $p = 0.0104$).

The relative expression of microRNA-590 was the highest in patients with AF without CHD. There was a 7.8-fold decrease in expression (95% CI 0.9–66.4, $p = 0.0658$) compared with the control group.

In patients with left atrial dilatation compared with the control group, there was a statistically significant 9.63-fold (95% CI 1.25–74.29) decrease in microRNA-590 expression ($p = 0.0313$) in the group of patients with AF without CHD. In addition, a 1.68-fold (95% CI 0.26–10.66) decrease in the expression of this microRNA was found in the within-group comparison between patients with and without left atrial dilatation, but this comparison did not show a significant statistical difference ($p = 0.5704$).

There was no statistically significant difference in the expression level of microRNA-590 between patients who underwent RFA and those who did not ($p = 0.3548$). The mean expression level in patients who underwent RFA was 2.32-fold lower (95% CI 0.37–14.44) than in those who did not.

There was a statistically significant association between the expression level of microRNA-590 and the development of AF recurrence after RFA. Patients who underwent recurrence had a mean expression level that was 15.69-fold lower (95% CI 2.16–113.95) than patients who did not ($p = 0.0083$). A decrease in microRNA-590 concentration was associated with a 1.74-fold increase in the likelihood of AF recurrence after RFA, independent of gender and age. The adjusted odds ratio was 1.74 (95% CI 1.16–3.43, $p = 0.0343$).

The expression level of microRNA-590 was compared between the different types of AF. The results showed that patients with persistent AF had an expression level 5.24 times lower than controls (95% CI 0.29–95.34, $p = 0.2412$), patients with paroxysmal AF had an expression level 6.99 times lower than controls (95% CI 1.1–44.53, $p = 0.0403$), and patients with persistent AF had an expression level 42.14 times (95% CI 1.84–962.63, $p = 0.0242$) lower than controls.

However, no statistically significant association of microRNA-590 expression level between the types of AF ($p = 0.3673$; Table 4) was found.

Table 4. Results of relative expression depending on the form of atrial fibrillation

AF form	ΔΔCT	FC [95% CI]	p
Paroxysmal/ permanent	–2,59	6,03 [95% CI 0,15–237,11]	0,4551
Persistent/ permanent	–3,01	8,05 [95% CI 0,15–433,62]	0,4081
Persistent/ paroxysmal	–0,42	1,34 [95% CI 0,11–16,31]	0,9556

Note: FC is the ratio of expression levels in the comparison groups; AF, atrial fibrillation; CI, confidence interval.

The mean expression level of patients with normosystolic AF was 15.24-fold lower (95% CI 1.99–116.71) than controls (p = 0.0113) and that with tachysystolic AF was 4.15-fold lower (95% CI 0.5–34.65, p = 0.178) than controls. There was no statistically significant association between the expression level of microRNA-590 and different forms of AF (p = 0.1407).

Discussion

Analysis of the relative expression level of microRNA-590 among groups revealed that a statistically significant decrease in expression was observed in patients with CHD and a more pronounced decrease was observed in patients with CHD + AF compared with the control group, suggesting a close relationship between the expression of microRNA-590 and the presence of CHD and comorbid pathology.

Increased activity of microRNA-590 leads to a significant proliferation of cardiomyocytes [1, 2]. This effect is achieved by suppressing the Hops gene, which regulates cardiac development [9]. Conversely, overexpression of Hops in tumor cell lines significantly reduces proliferation by arresting the cell cycle in G0/G1. Hops is involved in centrosome assembly and maintenance, and its knockdown causes genome instability [10]. The decrease in microRNA-590 activity level in the groups of patients with chronic CHD was likely due to the inhibition of Hops expression.

According to S. Fichtlscherer et al., microRNA-17, microRNA-20a, microRNA-21, microRNA-92a, and microRNA-590 are suppressed in chronic CHD [11]. Moreover, they concluded that the microRNA-106b/25 cluster, microRNA-17/92a cluster, microRNA-21/590-5p family, microRNA-126, and microRNA-451 signature can serve as biomarkers for the diagnosis of unstable angina [12], as was observed by M. Piccoli et al. [13].

A previous study revealed that microRNA-590 has an anti-atherosclerotic effect by inhibiting lipoprotein lipase expression in mouse macrophages [14]. The progression of atherosclerotic processes, including in coronary vessels, can occur because of decreased expression of microRNA-590. This may explain the low activity of microRNA-590 in patients with CHD or CHD-prone AF.

In this study, we found a significant decrease in the expression level of microRNA-590 in patients with dilatation of the left atrium and permanent AF, which may be associated with a slowing down of proliferation processes along with dilatation of heart chambers, which can lead to increased consumption of this microRNA and inversely dependent depression of its formation. In addition, cardiac microRNAs can be inhibited by various factors. For example, Shan et al. demonstrated an increase in collagen synthesis and fibrosis in the atria of dogs exposed to nicotine. These changes increased the risk of AF and were accompanied by a decrease in microRNA-590 and microRNA-133 expression. Similar changes were observed in smokers with AF [15].

Furthermore, we found that microRNA-590 expression was decreased in patients with recurrent rhythm disturbances after RFA. We did not find any data on microRNA-590 alteration under these conditions during our literature analysis, suggesting that such studies have not been conducted. On the basis of these findings, we can assume that these results are associated with the presence of arrhythmogenic myocardial remodeling and, consequently, increased myocardial fibrosis.

Thus, our study demonstrates that chronic CHD suppresses microRNA-590 expression, which regulates proliferative processes in the cardiac muscle. For the comorbid pathology of AF and CHD, the microRNA-590 activity is significantly decreased compared with that in the control group, suggesting depression of proliferation and remodeling processes under the influence of myocardial ischemia.

Atrial dilatation and persistent AF, as well as AF recurrence after RFA, decrease the relative expression of microRNA-590. This decrease may serve as a prognostic marker for these clinical features and the severity of AF.

The above results indicate that a decreased microRNA-590 expression may indicate disease progression and an increased expression may be a positive prognostic factor.

Conclusions

The combined pathology of CHD and AF, along with aggravating factors such as left atrial dilatation, persistent AF, and recurrence of rhythm disturbances after RFA, can result in decreased microRNA-590 expression levels.

Authors' contribution. D.D.L., patient selection, conduct of the study, analysis of results, writing and design of the article; S.D.M., supervision of the study; O.A.K., conduct of the study, analysis of results; R.S.T., conduct of the study.

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