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PLATELET miRNA EXPRESSION IN PATIENTS WITH STICKY PLATELET SYNDROME

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Abstract

Sticky platelet syndrome (SPS) is a disorder with familial occurrence and autosomal dominant trait characterized by platelet hyperaggregability in response to a low concentration of adenosine diphosphate (ADP) and/or epinephrine (EPI). The etiology of SPS may be associated with platelet microRNAs (miRNAs), which are considered as potential biomarkers of platelet function and antiplatelet therapy. We were monitoring the expression of platelet miRNAs in patients with laboratory diagnosed SPS and healthy controls. We have found a statistically significant increased expression of both miR-423-5p and miR-338-3p as well as a statistically significant decreased expression of miR-425-5p between the group of patients with diagnosed SPS type II and the group of healthy controls, which seems to be an interesting issue for a further research.

Key words: sticky platelet syndrome, miRNA, expression

INTRODUCTION

Sticky platelet syndrome is a thrombophilic thrombocytopathy defined as hyperaggregability in response to a low concentration of adenosine diphosphate (ADP) and/or epinephrine (EPI), while aggregability after other reagents is normal. Diagnostics is based on laboratory results of aggregation testing. According to the aggregation pattern, three types of the syndrome can be identified (Type I = hyperresponse after both inducers, Type II = hyperresponse after EPI alone, Type III = hyperresponse after ADP alone). Clinical manifestation is mostly associated with venous or arterial thrombosis. The first thrombotic event usually occurs before 40 years of age and without prominent acquired risk factors. Direct genetic background of this inherited thrombophilic disorder is not sufficiently known, but the genetic changes of platelet membrane receptors play a possible role in platelet activation and aggregation (1, 2).

An interesting issue for the research of SPS etiology seems to be platelet miRNAs. MiRNAs are small, non-coding RNAs, which are able to regulate cellular functions by specific gene modifications and they have been shown to modify the expression of platelet proteins. They are predicted to modulate many targets and they are involved in regulating various cellular processes. Circulating miRNAs can be used as diagnostic and prognostic biomarkers of vascular thrombosis affecting platelet reactivity as well as novel therapeutic targets. Various clinical studies have shown associations between circulating miRNA levels and platelet reactivity pathways or the recurring cardiovascular events (3,4,5).

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AIM

Monitoring the expression of chosen platelet miRNAs in patients with laboratory diagnosed SPS and considering their possible role in pathogenesis of SPS.

MATERIAL AND METHODS

Following population presents 40 samples of isolated platelets, 18 patients with SPS type I, median 32 years (5–52), 10 patients with SPS type II, median 30 years (13–45), and 12 healthy controls, median 31 years (22–54). For the analysis of samples we used light transmission aggregometry (LTA, Agg-RAM, Helena Laboratories, USA) established by Mammen (6) and Bick (7). The platelet function was evaluated by testing the aggregation in response to 2 low concentrations ADP and EPI (0.58 and 1.17 mmol/L). SPS I was confirmed by hyperaggregation in response to at least 1 low concentration of ADP and EPI. SPS II was confirmed by hyperaggregation in response to 2 low concentrations of EPI.

For platelet separation we used magnetic separation system MidiMACS™ Separator (MiltenyiBiotec, Zürich, Switzerland), together with superparamagnetic plastic particles (MicroBeads – MiltenyiBiotec GmbH, Bergisch Gladbach, Germany), which are conjugated with CD45 and CD235a antibodies for the removal of white blood cells and granulocytes from the samples. The CD45 MicroBeads were used for a positive selection or depletion of leukocytes from the peripheral blood and the CD235a MicroBeads were used for a positive selection or depletion of human erythroid cells. Unlabelled cells passed through the separator while magnetically labelled cells were retained within the separator. The number of cells was determined by the blood cell analyser (8).

MiRNA was isolated by High Pure miRNA Isolation Kit (Roche, Germany). For the measurement of chosen platelet miRNAs expression *(hsa-miR-221-3p, hsa-miR-33a-5p, hsa-let-7b-5p, hsa-let-7i-5p, hsa-miR-423-5p, hsa-miR-338-3p, hsa-miR-148a-3p, hsa-miR-22-3p, hsa-miR-425-3p, hsa-miR-29b-3p, hsa-miR-326, hsa-miR-18a-5p, hsa-miR-148b-3p, hsa-miR-331-3p, UniSP3, UniSp6)* we used a real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) with miRCURY® LNA® miRNA PCR Assays and PCR panels (Qiagen, Germany) exosome isolation kit. For normalization we used a panel for platelet miRNA consisting of *miR-148b, miR-151-3p, miR-18a, miR-28-5p, miR-29c, miR-331-3p*. We were monitoring the fold change DELTA, which is a fold gene miRNA expression compared to the control. The average value of the control equals 1. If the fold change DELTA is <1, miRNA expression is reduced and if the fold change is >1, the expression is increased.

All data we got from qRT-PCR were processed in QIAGEN GeneGlobe Data analysis center and statistical tests were performed in Microsoft Excel spreadsheet. The results with p-value < 0.05 were considered statistically significant (9).

RESULTS

We were monitoring the platelet miRNAs expression in patients with diagnosed SPS and healthy controls. We surveyed the statistically significant increased expression of both miR-423-5p (fold change 1.33; p=0.05) and miR-338-3p (fold change 1.32; p=0.05) as well as the statistically significant decreased expression of miR-425-5p (fold change 0.69; p=0.01) between the group of patients with SPS type II and the group of healthy controls (Tab.1).

The expression of miR-29b-3p in patients with SPS type II was significantly decreased, but this change was not statistically significant (p=0.38). In the group of patients with SPS type I we have not found any statistically significant changes of the platelet miRNAs expression .

Table 1 Statistically significant changes of platelet miRNAs expression

miRNA (mature ID)	Fold change DELTA		p-value		Comment
	SPS1	SPS2	SPS1	SPS2	
hsa-miR-423-5p	1.13	**1.33**	0.28	**0.05**	increased expression in SPS 2
hsa-miR-338-3p	1.07	**1.32**	0.33	**0.05**	increased expression in SPS 2
hsa-miR-425-3p	0.92	**0.69**	0.55	**0.01**	decreased expression in SPS 2
hsa-miR-29b-3p	0.81	0.49	0.89	0.38	

Abbr.: n – number, miRNA – microRNA, SPS – sticky platelet syndrome

DISCUSSION

The pathogenesis of sticky platelet syndrome remains unknown and the diagnosis is based on the laboratory results and clinical manifestation. Platelet microRNA may have a potential role as a biomarker of platelet hyperaggregability (9).

MiRNAs are non-coding RNAs which are able to modify the expression of platelet proteins influencing platelet activity. Circulating miRNAs have been suggested as diagnostic and prognostic biomarkers, which can be also used as therapeutic targets. MiRNAs modify the platelet proteins expression by targeting mRNAs, which alters their biochemical pathways. Some biochemical pathways are associated with drug response, which means they can impact on efficiency of antiplatelet therapy responsiveness. Platelet-related miRNAs have been suggested also as a biomarker for the assessment of antiplatelet therapy efficiency by various clinical studies. Antiplatelet therapy is recommended as a lifelong therapy for patients with SPS. Molecular mechanisms of platelet pathways are important for further studies, because a large number of patients requires antiplatelet treatment (3,10,11).

MiRNAs are rising to be the possible biomarkers for both cardiovascular diseases and cancer. They play a role in regulating the expression of many proteins involved in hemostasis and cancer and they may help to understand and predict the risk of venous thromboembolism (VTE). The increased expression of miR-423-5p have been shown to be a diagnostic biomarker of deep vein thrombosis (DVT). Its potential aim is mRNA in genes VEGF and eNOS. Unfortunately most of the studies used only small sample sizes and lacked external validation (12).

Recently we were monitoring the changes in expression of miR-96-5p, miR-126-3p, and miR-223-3p in platelets of 45 patients with diagnosed SPS and 30 healthy controls. We have not found any correlation between these 3 chosen miRNAs and the phenotype of SPS, but interestingly we have found a significantly increased expression of miRNA-96-5p in platelets of SPS patients with pregnancy complications ($p<0,01$) (9).

Our present study was monitoring the expression of chosen miRNAs in patients with SPS. Current results showed a statistically significant increased expression of both miR-423-5p and miR-338-3p as well as a statistically significant decreased expression of miR-425-5p between the group of patients with diagnosed SPS type II and the group of healthy controls. The expression of these 3 miRNAs can be associated with the pathogenesis of SPS and we consider it as an interesting issue for further clinical studies in larger population.

However, comparing the results of different studies has several limitations. The expression of miRNAs is being measured in different types of biological samples (platelet poor plasma, platelet rich plasma or isolated platelets) and different laboratory protocols are used, some

types of miRNAs expressed by platelets can be also released by other cells, platelet reactivity is measured by various incomparable methods and the results of studies depend on genetic variability of populations living in the different geographical regions. In future the standardization of measurement is needed to establish many practical aspects and to get comparable results (3).

CONCLUSION

MiRNAs play an important role in platelet function and reactivity pathways of platelets. Some of them can aim on mRNA coding proteins, which are associated with aggregation, thus they can regulate their expression. According to our results the increased expression of platelet miR-423-5p and miR-338-3p as well as the decreased expression of miR-425-5p seem to be an interesting issue for a further research and testing in larger group of patients with SPS.

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