Microarray analysis of circulating micro RNAs in the serum of patients with polymyositis and dermatomyositis reveals a distinct disease expression profile and is associated with disease activity

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Abstract

Objective

The aim of this study was a large scale investigation of myositis-associated circulating miRNA molecules and also determination of expression of these candidate molecules in relation to clinical activity of myositis.

Methods

RNA, containing also miRNAs, was isolated from sera of 28 patients suffering from idiopathic inflammatory myopathies (IIM) and 16 healthy controls. Expression of miRNAs was determined using a miRNA microarray method. Statistical analysis of miRNA expression was carried out using Arraystar software.

Results

Our results showed 23 significantly differentially expressed miRNAs. Six miRNAs were differentially expressed in IIM compared to healthy controls. In dermatomyositis (DM) we found 3 and in polymyositis (PM) 6 differentially expressed miRNAs compared to controls. Three miRNAs were up-regulated in patients with highly active disease compared to patients with low disease activity. Furthermore, we found 26 significantly differentially expressed miRNAs in SLE patients compared to IIM, DM and PM patients.

Conclusion

This is the first study that comprehensively describes expression levels of circulating miRNAs in serum of patients suffering from IIM. It can be expected that some of these deregulated miRNA molecules are involved in aetiology of IIM and may potentially serve as molecular markers for IIM development or for monitoring of disease activity.

Key words

circulating microRNA, idiopathic inflammatory myopathies, serum

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Introduction

Idiopathic inflammatory myopathies (IIM) belong to a group of autoimmune diseases characterised by chronic muscle inflammation that can lead to a dysfunction and/or destruction of muscle cells. The aetiology of these diseases is unknown and recent findings suggest that both, immune and non-immune mechanisms are involved in the pathogenesis of myositis (1, 2). Moreover, in different subsets of myositis, different molecular pathways might predominate (3). Differential diagnosis may be complicated in IIM; diagnostic errors, late detection of the disease and prolonged treatment initiation can significantly worsen the prognosis and result in irreversible tissue damage (4). Current data suggest that the presence of MHC haplotype A1-B8-Cw7-DRB1*0301/DQA1*0501 is a strong genetic risk factor for IIM development in a large subset of white patients (5, 6). Besides known genetic risks located within the MHC complex, also epigenetic regulations including changes in miRNAs expression profiles have been implicated recently in the pathogenesis of autoimmune diseases (7-9).

MicroRNAs (miRNAs) are small, non-coding, evolutionarily conserved RNA molecules posttranscriptionally regulating gene expression. Epigenetic mechanism of RNA interference (RNAi) influences the stability and translational efficiency of the target mRNA. MiRNAs can be found in most organisms and they form about 1–2% of eukaryotic genomes. They have the ability to control the expression of approximately half of the protein-coding genes (10). MicroRNA expression profiles are typically tissue- and cell- specific and are strictly controlled (11). Aberrant expression patterns are included in the pathogenesis of a wide range of diseases including systemic autoimmune diseases (12). A specific group of miRNAs biomarkers represent extracellular circulating miRNAs (c-miRNAs) which may provide a new insight also into the pathogenesis of autoimmune diseases (13). Growing evidence indicates that c-miRNAs may serve as biomarkers to assess pathophysiological status (14). Indeed, in muscular dystrophies significantly changed c-miRNAs levels have been described as well as in patients with chronic obstructive pulmonary disease, who often exhibit reduced muscle fibre size (15). It was recently suggested that c-miRNA may move into other organs or muscle itself and may regulate their functions (16). Several studies investigated expression of miRNAs in the muscle tissue (13, 17-19) and few also looked at serum levels of miRNAs (20-22). A single miRNAs were selected for each of these studies and, so far, no report analysed comprehensive status of miRNAs in patients with polymyositis and dermatomyositis (8, 23-25). Therefore in the current study we aimed to investigate myositis associated circulating miRNA molecules on a large scale and also to determine the relation of expression of these candidate molecules to clinical course of myositis.

Methods

Group of patients

Twenty-eight patients suffering from idiopathic inflammatory myopathies (IIM), who fulfilled Bohan and Peter criteria for the diagnosis of polymyositis (PM) or dermatomyositis (DM) were investigated in this study. Basic characteristics of patients with myositis are shown in Table I. A group of healthy individuals (7 females, 9 males; mean age 41.3±11.3 years) and a group of patients with systemic lupus erythematosus (16 females, mean age 44.1±15.1) served as controls.

Disease activity was assessed using visual analogue scale (Physician's Global Assessment [PGA]) (range 0–100 mm). For the purpose of this study the cut-off for disease activity was selected at 40. Patients below this cut-off were considered to have low activity; patients above the cut-off were assigned as having a highly active disease. Written informed consent was obtained from all participants, and the study was approved by local ethics committee.

Collection of biologic material and RNA extraction

Peripheral blood samples were collected into vacuum plastic tubes containing spray-coated silica. Samples were handled according standardised laboratory

Table I. Demographic and basic clinical data of patients with idiopathic inflammatory myopathies.

Clinical data of patients with idiopathic inflammatory myopathies	IIM
Sex (female/male)	20/8
Age (yrs)	
Mean ± SD	54.5 ± 15.0
Diagnosis	
DM (n)	14
PM (n)	14
Disease duration	
Mean ± SD	8.2 ± 4.0
Physician's Global Disease Assessment [a]	35.2 ± 25.4
- high global disease activity >40mm [b]	n=18; 51.9 ± 13.8
- low global disease activity <40mm [b]	n=10; 5.1 ± 3.0
HAQ	1.2 ± 0.9
CK	24.6 ± 42.7
LDH	7.3 ± 5.5
CRP	15.2 ± 31.8
Disease activity[a]	
Constitutional	11.5 ± 16.4
Cutaneous	9.7 ± 16.8
Skeletal	4.8 ± 10.6
Gastrointestinal	10.5 ± 17.4
Pulmonary	15.9 ± 21.2
Cardiovascular	3.6 ± 9.8
Extramuscular	21.6 ± 21.8
Muscle	36.7 ± 30.2

[a]Shown as mean ± SD (in mm on visual analogue scales). IIM: idiopathic inflammatory myopathy; [b]number of patients, mean±SD; PM: polymyositis; DM: dermatomyositis. Upper normal limits: CK 2,85 μkat/l; LDH 4.13 μkat/l; CRP 5 mgl.

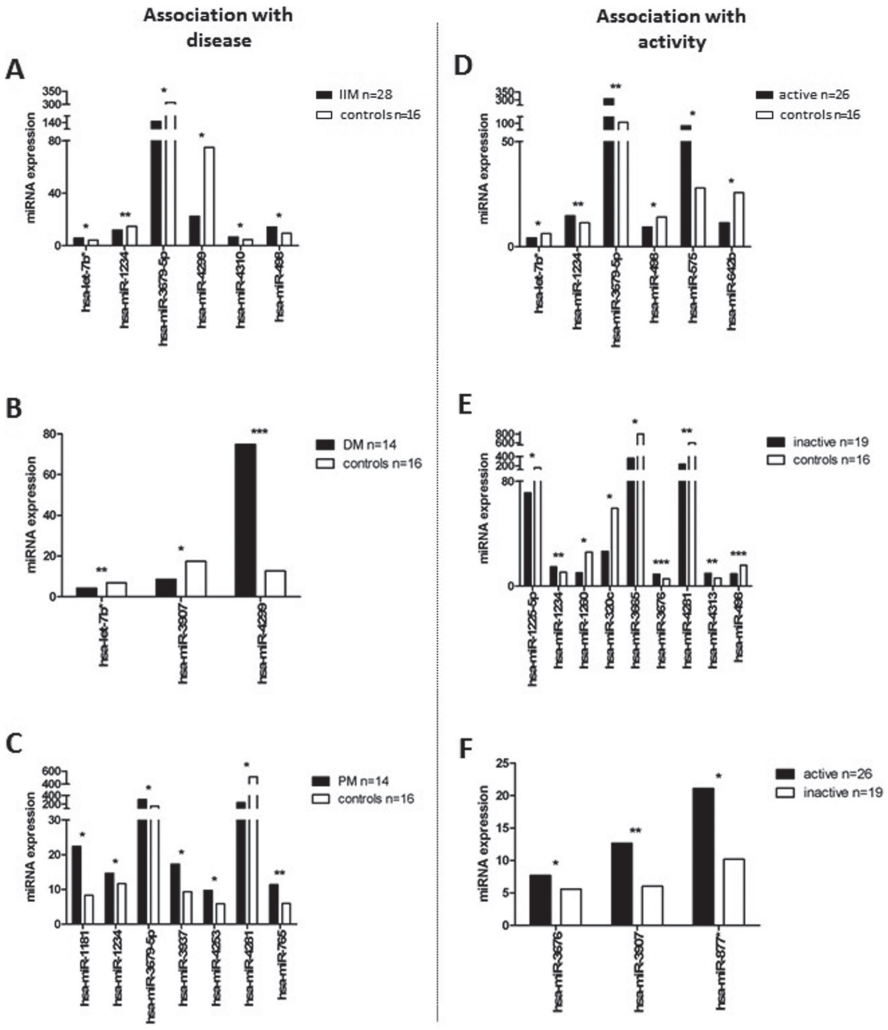

Fig. 1. Association of microRNA expression pattern with IIM diagnoses and with disease activity. Six microRNAs are significantly differentially expressed in patients with myositis compared to healthy controls.
A. After we divided the patients according to their diagnosis into two groups (DM and PM), we found in total 3 differentially expressed miRNAs in DM patients compared to controls.
B. and 7 microRNAs differentially expressed in PM patients compared to controls.
C. The analysis of microRNA expression pattern and relation to the disease activity revealed additional molecular associations. Six miRNAs were differentially expressed in patients with active stage of the disease when compared to healthy controls.
D. and 9 miRNAs were differentially expressed in patients with inactive stage of the disease when compared to controls.
E. Furthermore, comparison of microRNA expression profile between patients with active and inactive stage of the disease showed 3 miRNAs that are significantly differentially expressed.
F. The disease activity was assessed using Physician's Global Disease Assessment scale. For the purpose of this study the cut-off for disease activity was selected at 40. Patients below this cut-off were considered to have lower or no activity, patients above the cut-off were assigned as having active disease. Expression levels are displayed as average expression values from all patients within one group. *P*-values were corrected using FDR (Benjamini Hochberg) method. $^{*}p<0.05$; $^{**}p<0.001$; $^{***}p<0.0001$.

procedures and were processed within 4 hours after blood withdrawal.

Isolation of RNA from sera, containing also miRNAs, was performed according to Filková *et al*., 2013 (26). In brief, 500 μl of individual serum sample was homogenised with 500 μl of Trizol® LS reagent (Life Technologies, USA) and incubated for 5 minutes at room temperature. The samples were cleared by centrifugation at 12,000 × g for 10 minutes at 4°C. The supernatant containing RNA was then processed 3 times by acid phenol-chloroform (Life Technologies, USA) extraction and aqueous phase was separated by centrifugation at 12,000 × g for 5 minutes at 4°C. RNA was precipitated by adding of 100 μg of RNase-free glycogen (Roche Diagnostics, Germany) and 100% isopropanol, incubated for 10 minutes at room temperature with subsequent centrifugation at 12,000 × g for 10 minutes at 4°C. RNA pellet was then washed with 75% ethanol, spin at 7,500 x g for 5 minutes at 4°C and air dried. The extracted RNA was dissolved in RNase-free water. The quality and quantity of extracted RNA samples were controlled using the NanoDrop 2000 (Thermo Fisher Scientific, USA) and Bioanalyzer 2100 with the Small RNA Kit (Agilent Technologies, USA).

Determination of disease specific miRNA expression signature using the microarray technology

Expression of miRNAs was determined using a single-channel platform

Table II. Differentially expressed miRNAs in patients with IIM compared to healthy controls.

SystematicName	Myositis	Controls	Fold change	*p*-value
hsa-miR-1234	11,822	14,659	-1,24	0,0078
hsa-miR-498	14,159	9,328	1,517	0,0113
hsa-miR-3679-5p	141,668	307,403	-2,169	0,0152
hsa-miR-4299	22,444	74,922	-3,338	0,0255
hsa-let-7b*	5,874	4,251	1,381	0,0412
hsa-miR-4310	6,542	4,537	1,442	0,0451

Relative expression data of patients (myositis) and controls, fold change and *p*-values are shown here. $p<0.05$ was considered as significant. P-values were corrected using FDR (Benjamini Hochberg) method.

of 8x60K high density human miRNA microarray method (Agilent Technologies, USA). Three hundred nanograms of total RNA were used as a starting material to prepare cDNA. Total RNA samples were dephosphorylated, 3´ end- labeled with Cy3-pCp, purified on Micro Bio-Spin columns, dried, and hybridised to the microarrays. The hybridisation was performed for 20 hours at 55°C. After washing, Cy3 was detected by one-color scanning using a DNA microarray scanner type G2505B (Agilent, USA) at 5 micron resolution. Scanned image files were visually inspected for artefacts. The data were extracted from the scanned images using Feature Extraction software (Agilent Technologies, USA).

Statistical analysis

Statistical analysis of miRNA expression was carried out using Arraystar software (Lasergene, USA). The signal intensities of the samples were transformed into $\log^2$-ratio data. The array data were normalised by the averaging summarisation of global medians across all arrays. The Student's *t*-test was used to identify differentially expressed miRNA molecules. A miRNA was defined as being differentially expressed if $p<0.05$ was observed between two data sets. *P*-values of the entire analysis were corrected using FDR (Benjamini Hochberg) method.

Results

Idiopathic inflammatory myopathies (IIM) have a distinct miRNA expression signature in serum

The miRNA microarray used in this study could analyse 1,673 human microRNA molecules. Six miRNAs (let-7b*, miR-1234, miR-3679-5p, miR-4299, miR-4310, miR-498) had significantly different expression levels in serum of IIM patients in comparison with healthy controls ($p<0.05$). The expression of miR-1234, miR-3679-5p, and miR-4299 was significantly down-regulated and the expression of let-7b*, miR-4310, and miR-498 was up-regulated in the serum of patients in comparison with control samples (Fig. 1A) (Table II).

The analysis of serum miRNA content in patients with DM revealed a differential expression of 3 miRNAs; one (miR-4299) was down-regulated and two (let-7b*, miR-3907) were up-regulated in DM compared to healthy controls ($p<0.05$) (Fig. 1B). Similarly, when we compared PM patients with healthy controls we found that 6 miRNAs (miR-1181, miR-1234, miR-3679-5p, miR-3937, miR-4253, miR-765) were significantly down-regulated and one miRNA (miR-4281) was up-regulated in PM patients ($p<0.05$) (Fig. 1C). No difference was found in serum miRNA expression when PM and DM patients were compared.

After combining both, $p<0.05$ and fold-change >1.5, we found three differentially expressed miRNAs (hsa-miR-498, hsa-miR-3679-5p and hsa-miR-4299) in all IIM patients compared to controls; 6 miRNAs (hsa-miR-1181, hsa-miR-3679-5p, hsa-miR-3937, hsa-miR-4253, hsa-miR-4281 and hsa-miR-765) in PM and 3 miRNAs (hsa-let-7b*, hsa-miR-3907 and hsa-miR-4299) in DM were differentially expressed when compared to healthy controls.

Differential expression of several miRNAs is associated with disease activity

Three miRNAs (miR-3676, miR-3907, miR-877*) were significantly up-regulated in patients with highly active disease (defined as physician's global activity >40 mm) compared to patients with low disease activity ($p<0.05$) (Fig. 1F). When compared with healthy controls, 6 miRNAs were differentially expressed in patients with highly active disease; three miRNAs (miR-1234, miR-3679-5p, miR-575) were down-regulated and 3 miRNAs (let-7b*, miR-498, miR-642b) were up-regulated in patients ($p<0.05$) (Fig. 1D). In patients with low or inactive disease 3 miRNAs (miR-1234, miR-3676, miR-4313) were down-regulated and 6 miRNAs (miR-1225-5p, miR-1260a, miR-320c, miR-3665, miR-4281, miR-498) up-regulated when compared to healthy controls ($p<0.05$) (Fig. 1E).

Furthermore, we made a comparison of DM and PM in active and inactive patients. Three miRNAs (hsa-let-7b*, hsa-miR-548d-5p and hsa-miR-4299) were found to be differentially expressed in active DM compared to active PM patients ($p<0.05$). One miRNA (hsa-miR-3648) was found to be up-regulated in inactive DM compared to inactive PM patients ($p<0.05$).

The overview of expression data of all analysed miRNA molecules is shown in Table III.

Furthermore, after taking into consideration both, $p<0.05$ and fold-change >1.5, two miRNAs (hsa-miR-3907 and hsa-miR-877*) were found to be differentially expressed in patients with highly active disease compared to patients with low disease activity. When compared with healthy controls, four miRNAs (hsa-miR-3679-5p, hsa-miR-498, hsa-miR-575 and hsa-miR-642b) were differentially expressed in patients with highly active disease and 8 miRNAs (hsa-miR-3676, hsa-miR-498, hsa-miR-4313, hsa-miR-4281, hsa-miR-3665, hsa-miR-1225-5p, hsa-miR-320c and hsa-miR-1260) were differentially expressed in patients with low or inactive disease.

Association of biological pathways regulated by detected miRNAs

An analysis of the associated biological pathways regulated by the detected miRNAs revealed that among all 23

Table III. Overview of microRNA expression in different analyses.

miRNA	patients *vs.* controls	PM *vs.* DM	PM *vs.* controls	DM *vs.* controls	active *vs.* inactive	active *vs.* controls	inactive *vs.* controls	DM_act *vs.* PM_act	DM_inact *vs.* PM_inact
hsa-let-7b*	↑			↑		↑		↑	
hsa-miR-1181			↓						
hsa-miR-1225-5p							↑		
hsa-miR-1234	↓		↓			↓	↓		
hsa-miR-1260							↑		
hsa-miR-320c							↑		
hsa-miR-3648									↑
hsa-miR-3665							↑		
hsa-miR-3676					↑		↓		
hsa-miR-3679-5p	↓		↓			↓			
hsa-miR-3907				↑	↑				
hsa-miR-3937			↓						
hsa-miR-4253			↓						
hsa-miR-4281			↑				↑		
hsa-miR-4299	↓			↓				↓	
hsa-miR-4310	↑								
hsa-miR-4313							↓		
hsa-miR-498	↑					↑	↑		
hsa-miR-548d-5p								↓	
hsa-miR-575						↓			
hsa-miR-642b						↑			
hsa-miR-765			↓						
hsa-miR-877*					↑				

miRNAs found to be significantly differentially expressed, 11 (48%) (miR-1260, miR-320c, miR-498, miR-575, miR-765, miR-3665, miR-3679-5p, miR-3907, miR-4253, miR-4281, let-7b*) are predicted to regulate immune response; 4 (17%) miRNAs (let-7b*, miR-1225-5p, miR-498, miR-765) are related to pathways of actin and myosin; and 2 (9%) miRNAs (miR-1225-5p, miR-3937) are known to play a role in autoimmune diseases. Stat3 protein is probably regulated by miR-1234 (27). MiR-1181 plays a vital role in inhibiting cancer stem cell-like phenotype in pancreatic cancer (28) and miR-642b was among the three serum markers identified with high diagnostic accuracy for early stage of pancreatic cancer (29).

MiRNA genes are distributed across chromosomes either individually, or in clusters. A miRNA cluster is a group of miRNA genes located within a short distance on a chromosome. Based on the miRBase database (http://www.mirbase.org) definition, clustered miRNAs are a group of miRNA genes located within 10 Kb of distance on the same chromosome. We examined the genomic location of all of the 21 miRNA molecules that were differentially expressed in patients with IIM. We have found that 4 of the differentially expressed miRNAs were located in the known miRNA clusters and 16 miRNAs were individually distributed. We did not find any 2 or more miRNAs that would locate into the same cluster.

MicroRNA expression pattern found in IIM is different from serum miRNAs in SLE

In order to see whether the significantly differentially expressed miRNA molecules found in IIM patients are disease specific, we have performed a similar analysis in SLE patients (Table IV). The microarray expression analysis showed that the expression of 19 miRNA molecules was significantly changed in SLE patients compared to healthy controls ($p<0.05$). Of them, 6 miRNAs (miR-1281, miR-1825, miR-3679-5p, miR-3907, miR-4313, miR-575) were down-regulated and 13 miRNAs (miR-22, miR-24, miR-33b*, miR-3610, miR-3663-3p, miR-3937, miR-4257, miR-4271, miR-4298, miR-584, miR-642b, miR-671-5p, miR-4306) were up-regulated in SLE patients compared to controls. One miRNA (miR-3679-5p) was down-regulated in SLE patients similarly as in sera of patients with IIM compared to controls. The remaining 18 molecules differentially expressed in SLE were not differentially expressed in IIMs, when both compared to healthy controls.

Of the 19 differentially expressed miRNAs in SLE, 15 molecules (hsa-miR-3679-5p, hsa-miR-671-5p, hsa-miR-4271, hsa-miR-575, hsa-miR-584, hsa-miR-4298, hsa-miR-642b, hsa-miR-22, hsa-miR-3937, hsa-miR-3663-3p, hsa-miR-4257, hsa-miR-3907, hsa-miR-3610, hsa-miR-24 and hsa-miR-4306) have reached fold-change >1.5.

Further analysis determining the expression of miRNA molecules in SLE compared to IIM, DM and PM patients was performed. Nineteen miRNAs were significantly differentially expressed in SLE patients compared to IIM patients ($p<0.05$) (Fig. 2; Table V). Only two of them (miR-1234 and miR-3679-5p) were similarly regulated when considering the results obtained from SLE *vs.* IIM and IIM vs controls comparisons. Ten miRNAs were found to be differentially expressed in SLE patients compared to DM patients and 20 miRNAs were found to be differentially expressed in SLE patients compared to PM patients.

The overview of expression data of all analysed miRNAs is shown in Table V. After combining both, $p<0.05$ and fold-change >1.5, we found 16 miRNAs (hsa-miR-584, hsa-miR-671-5p, hsa-miR-4271, hsa-miR-3937, hsa-miR-4257, hsa-miR-3907, hsa-miR-320b, hsa-miR-3679-5p, hsa-miR-1290, hsa-miR-4298, hsa-miR-575, hsa-miR-1275, hsa-miR-939, hsa-miR-197, hsa-miR-3620 and hsa-miR-3610) to be differentially expressed in SLE patients compared to IIM patients.

Discussion

Idiopathic inflammatory myopathies are autoimmune diseases characterised by chronic muscle inflammation that can lead to a dysfunction and/or destruction of muscle cells. It is known that some microRNAs play role in muscle differentiation and thus also epigenetic regulations may be implicated in

Table IV. Differentially expressed miRNAs in patients with SLE compared to healthy controls.

SystematicName	SLE	Controls	Fold change	*p*-value
hsa-miR-3679-5p	61,674	307,403	4,984 down	7,23E-06
hsa-miR-671-5p	52,557	16,085	3,267 up	0,00011
hsa-miR-4271	56,48	23,365	2,417 up	0,00048
hsa-miR-1281	8,179	11,299	1,381 down	0,00061
hsa-miR-575	14,369	81,02	5,638 down	0,00155
hsa-miR-584	41,569	9,63	4,316 up	0,00196
hsa-miR-4298	39,395	14,402	2,735 up	0,00213
hsa-miR-642b	32,972	11,337	2,908 up	0,00301
hsa-miR-22	47,177	16,362	2,883 up	0,00488
hsa-miR-3937	39,641	17,316	2,289 up	0,00722
hsa-miR-3663-3p	112,625	33,638	3,348 up	0,00821
hsa-miR-1825	7,677	9,422	1,227 down	0,0115
hsa-miR-4313	8,09	9,701	1,199 down	0,0159
hsa-miR-4257	18,579	10,438	1,780 up	0,0179
hsa-miR-3907	3,944	8,643	2,191 down	0,0218
hsa-miR-3610	42,85	16,43	2,608 up	0,0225
hsa-miR-33b*	6,498	5,339	1,217 up	0,0399
hsa-miR-24	18,46	8,65	2,133 up	0,0481
hsa-miR-4306	12,427	5,173	2,402 up	0,0492

Relative expression data of patients (SLE) and controls, fold change and *p*-values are shown here. $p<0.05$ was considered as significant. *p*-values were corrected using FDR (Benjamini Hochberg) method.

Table V. Differentially expressed miRNAs in patients with SLE compared to IIM, DM and PM.

miRNA	SLE *vs*. IIM	SLE *vs*. DM	SLE *vs*. PM
hsa-miR-1224-5p		↓	
hsa-miR-1234	↓		↑
hsa-miR-1275	↑	↓	
hsa-miR-1280			↓
hsa-miR-1281	↑		↓
hsa-miR-1290	↓	↑	
hsa-miR-1825	↑		↓
hsa-miR-197	↑		
hsa-miR-2276			↑
hsa-miR-320b	↑	↓	
hsa-miR-320d		↓	↑
hsa-miR-3610	↓		↑
hsa-miR-3620	↓		↑
hsa-miR-3663-3p			↑
hsa-miR-3679-5p	↑		↓
hsa-miR-3907	↑	↓	↓
hsa-miR-3937	↓		↑
hsa-miR-4253			↑
hsa-miR-4257	↓	↑	↑
hsa-miR-4271	↓	↑	↑
hsa-miR-4298	↓		↑
hsa-miR-575	↑		↓
hsa-miR-584	↓	↑	↑
hsa-miR-601			↓
hsa-miR-671-5p	↓	↑	↑
hsa-miR-939	↑		

Relative expression data of significantly differentially expressed miRNAS of patients with SLE and IIM, fold change and *p*-values are shown here. $p<0.05$ was considered as significant.

pathogenesis of this disease. Recently, growing evidence has shown that some miRNAs persist in circulation. In 2007, Valadi *et al*. demonstrated that miRNAs can be taken up into intracellular vesicles and afterwards released into circulation. Many studies have shown the existence of circulating miRNAs (c-miRNAs) in various human body fluids (serum, plasma, breast milk, urine, saliva, etc.) (11). A significant relation between changed expression of circulating microRNAs and many autoimmune diseases has been described (30).

In our study, we have found in total 21 miRNA molecules that were significantly differentially expressed in patients when compared to control cohorts. Most of these miRNAs (miR-1234, miR-4299, miR-1181, miR-4281, miR-765, miR-575, miR-642b, miR-1225-5p, miR-1260, miR-320c, miR-3665, miR-877, miR-498) were studied in diseases and medical conditions including cancer, cardiovascular complications, or infections. So far, none of these miRNAs were found to be differentially expressed in autoimmune diseases.

To date, there are only few studies that describe the relationships between miRNAs and IIM, mostly in DM. Shimada *et al*. (25) hypothesised that if the miR-21 expression in the muscle tissues is significantly elevated in DM patients then serum miR-21 levels might be a potential biomarker for diagnosis and monitoring of disease activity in DM patients. Their results showed that serum levels of miR-21 were up-regulated in DM patients and related to the disease activity. This indicated that serum miR-21 might be involved in the pathogenesis of this disease. Oshikawa *et al*. (23) analysed miR-7 levels in serum of patient suffering from IIM. They have demonstrated that serum levels of miR-7 are specifically down-regulated in DM patients as well as in PM patients or CADM patients. However, levels of miR-7 were not decreased in patients with other autoimmune diseases such as SSc. Thus they indicate that serum miR-7 levels could possibly be used as a diagnostic marker for PM/DM. Results of our study could not support these findings, since both miR-21 and miR-7 were not differentially present in the serum of patients and controls or were present in different quantities in patients with active or inactive disease. Different miRNA molecules were found in analyses of patients with SLE. Wang *et al*. (31) investigated the levels of miR-146a and miR-155 in the serum of SLE patients. This was the first description of circulating miRNAs as biomarkers. They revealed that serum levels of miR-146a and miR-155 were decreased in SLE, and miR-146a was inversely associated with proteinuria

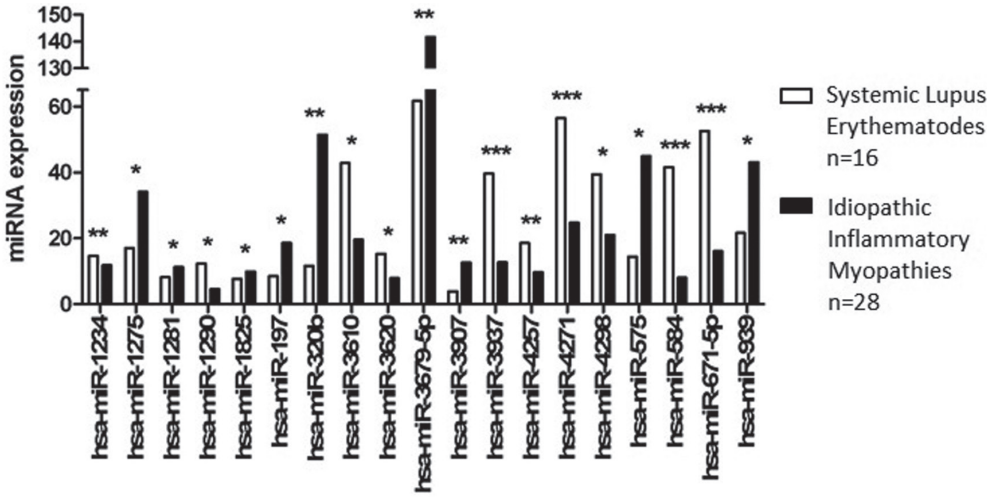

Fig. 2. Expression of microRNAs in different analyses using high density human miRNA microarray. In total, nineteen microRNA molecules have been found to be significantly differentially expressed in patients with myositis when compared to patients suffering from SLE. Expression levels are displayed as average expression values from all patients within one group. *P*-values were corrected using FDR (Benjamini Hochberg) method. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

and SLE Disease Activity Index (SLEDAI). These findings indicated that serum miR-146a and miR-155 may participate in the pathophysiology of SLE. The same group later conducted a pilot study in order to find a specific miRNA signature of SLE. They found that the serum levels of 6 miRNAs (miR-200a, miR-200b, miR-200c, miR-429, miR-205 and miR-192) were decreased in SLE patients compared to HCs (32). In 2012, Wang *et al*. (21) identified circulating miRNAs that were altered specifically in patients with SLE compared with RA and HCs. Based on these results 8 miRNAs were selected for continued clinical study. These molecules may be important regulators of immune cell development, playing vital roles in the inflammatory response and as key players in the pathogenesis of SLE (33). Carlsen *et al*. (34) found 7 miRNAs that were significantly differentially expressed in patients with SLE compared to patients with RA and healthy controls. MiR-142-3p and miR-181a were increased; miR-106a, miR-17, miR-20a, miR-203 and miR-92a were decreased in patients suffering from SLE.

None of these miRNAs were revealed as significantly regulated in our study. The comparison of sera from patients with SLE to healthy controls showed 19 miRNA molecules (miR-1281, miR-1825, miR-3679-5p, miR-3907, miR-4313, miR-575, miR-22, miR-24, miR-33b*, miR-3610, miR-3663-3p, miR-3937, miR-4257, miR-4271, miR-4298, miR-584, miR-642b, miR-671-5p, miR-4306) with significantly changed expression. Molecules found to be significant for IIM patients differed from molecules found to be deregulated in SLE patients except one (miR-3679-5p). This means that the expression of circulating miRNAs in the serum is largely different in the two diseases, perhaps forming a specific pattern for the respective disease. MiR-3679-5p was found down-regulated in the serum of patients with IIM, particularly in PM and active disease, as well as it was significantly lower in the serum from patients with SLE. There is not yet published information available on the role of miR-3679-5p in human organism; a number of predicted targets were identified for this miRNA with a putative role of targeted genes in immune system and in different molecular and structural reactions.

We have analysed also the expression of miRNA according to the disease severity. Disease activity was measured in every patient, cut-offs were set and patients with high and low or no activity were compared. Three miRNAs were found to be significantly differentially expressed (miR-3676, miR-3907, miR-877*) between patients with high and low activity. It remains to be determined for the future whether the levels of these miRNAs fluctuate longitudinally with the changes of disease activity and if so, whether they can be used as biomarkers for the disease. There were also a number of miRNAs differentially expressed in patients with active or inactive disease in comparison with healthy controls. It will be interesting to find out any relations with other disease parameters, particularly disease damage, where we do have a very limited spectrum of tools for muscle damage evaluation.

In summary, we provide here the first analysis of circulating serum miRNA gene expression profile in patients suffering from IIM. The results of this study indicate that the miRNA expression profile in serum of patients suffering from IIM is disease specific and creates a disease specific signature. It is expectable that some of these deregulated miRNA molecules are involved in aetiology of IIM. Additionally, we have shown that the detection of miRNA molecules in human serum is fast and easy approach in laboratory medicine. Therefore deregulated microRNA molecules shown in this work can potentially serve as molecular markers for IIM development or for monitoring of disease activity.

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