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Specific autoantibody profiles and disease subgroups correlate with circulating micro-RNA in systemic sclerosis

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Key messages

- SSc has a highly variable clinical presentation that may reflect different disease entities.
- Specific plasma miRNA profiles are present in the different SSc subtypes and autoantibody groups.
- Circulating micro-RNAs are epigenetic regulators that may be important for the pathogenesis of SSc.

Abstract

Objective. To evaluate the expression profiles of cell-free plasma miRNAs in SSc and to characterize their correlation with disease subgroups (lcSSc and dcSSc) and with autoantibody profiles.

Methods. Using quantitative RT-PCR, the abundance of 45 mature miRNAs in plasma was determined in 95 patients (lcSSc = 63; dcSSc = 32), representing the following autoantibody subgroups: ACA, anti-DNA topoisomerase I, anti-RNA polymerase III and anti-U1-ribonucleoprotein. MiRNA data were correlated with clinical and paraclinical data. Multiple regression was used to model membership of the lcSSc, dcSSc and autoantibody subgroups, based on miRNA expression profiles.

Results. Thirty-six miRNAs were measurable in all samples. Four (miRNA-223, -181b, -342-3p and -184) were differently expressed in lcSSc and dcSSc (false discovery rate < 0.05). Ten miRNAs exhibited statistically significantly different levels in one or more autoantibody groups, and five (miRNA-409, -184, -92a, -29a and -101) remained significant after correction for multiple comparisons. Multiple regression models accurately predicted ACA and anti-DNA topoisomerase I antibody–positive patients (area under the curve (AUC) = 0.97 and 0.93, respectively) as well as membership of the dcSSc and lcSSc groups (AUC = 0.88).

Conclusion. Circulating miRNA profiles differ between lcSSc and dcSSc patients and between patients with different autoantibodies. This is the first time autoantibody profiles, disease phenotypes and plasma miRNA profiles have been shown to correlate in an autoimmune disease. The data support a pathobiological role of miRNAs because specific miRNAs associate with autoantibody profiles of known diagnostic and prognostic value.

Keywords

systemic sclerosis, scleroderma, micro-RNA, autoantibody, plasma

Introduction

SSc is a rare but serious autoimmune disease of unknown cause. It is characterized by excessive fibrosis in connective tissues, vasculopathy and immunological anomalies such as the occurrence of disease- and prognosis-associated circulating autoantibodies [1]. SSc is heterogeneous in its presentation. Based on the extent of skin involvement, it is divided into lcSSc and dcSSc [2]. Both groups are characterized by mutually exclusive autoantibody profiles, with a preponderance of ACA in lcSSc and anti-DNA topoisomerase I (scl-70, ATA) or anti-RNA polymerase III (ARA) autoantibodies in dcSSc patients. A subgroup of lcSSc has anti-U1 ribonucleoprotein (RNP) autoantibodies. This antibody may indicate overlap with other CTDs.

miRNAs are recently discovered post-transcriptional regulators of protein expression [3], and miRNA may therefore provide disease diagnostic and activity markers [4]. In a Danish cohort, we have previously shown that specific expression profiles of circulating miRNAs associate with the SSc diagnosis compared with healthy controls and SLE patients [5]. However, SSc is a heterogeneous disease with a broad range of symptoms, disease severities and outcomes. It is not yet known whether circulating plasma miRNAs vary between SSc patients with different clinical phenotypes or between autoantibody-defined subgroups of SSc.

In the present study, we therefore examined a Swedish cohort of SSc patients to explore correlation of plasma miRNAs with phenotype and autoantibody profiles. Our hypothesis is that patient subsets with different prognoses, for example, represented by different autoantibody profiles, are characterized by specific miRNA profiles.

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Patients and methods

Patients

We included 95 SSc patients (82 women, 13 men) who received care at the Systemic Sclerosis Unit, Department of Rheumatology, Skåne University Hospital in Lund, Sweden. The patients were enrolled between 31 March 1989 and 1 January 2012. The clinical data are shown in Table 1. The patients were chosen at random from the whole cohort in order to fulfil the distribution of four groups (n = 20 each for ATA/ARA/RNP and n = 35 for ACA) defined

by the presence of specific autoantibodies that are relevant for SSc and regularly analysed in our cohort. No patient had more than one type of autoantibody detected. The patients had a median (interquartile range, IQR) age of 51 years [interquartile range (IQR) 43–63] and a median disease duration of 20 months (IQR 10–37). All patients fulfilled the criteria of a definitive diagnosis of SSc according to the ACR [6] or the 2013 classification criteria for SSc [7]. Sixty-three patients (59 women, 4 men) had lcSSc, and 32 (23 women, 9 men) fulfilled the criteria for dcSSc [2]. Twenty-five patients were on treatment with median 10 mg (IQR 5–15) of prednisolone daily. Seven patients were on treatment with CYC. Three patients received MMF. One patient each received penicillamine, MTX, AZA or HCQ. Four patients with RNA polymerase III antibodies were on dialysis. The Regional Ethics Review Board Lund, Sweden, approved the study (Dnr590/2008, Dnr2010/32), and written informed consent was obtained from all patients according to the Declaration of Helsinki.

Clinical assessment

Clinical and routine paraclinical data were obtained under identical conditions and as close as possible (usually within 1 week) to the blood sampling date as previously described [8,9]. The modified Rodnan skin score was evaluated by standardized palpation of the skin [10]. Oesophageal involvement was defined as distal hypomotility on cine-radiography. Radiological lung involvement was defined as basal interstitial fibrosis on plain chest X-ray or high-resolution CT [9]. Lung function tests included assessment of vital capacity by dry spirometry and diffusing capacity for carbon monoxide by single-breath test. Values are listed as percentage of predicted (p%). Cardiac involvement was assessed by radiological examination, 12-lead ECG and Doppler cardiography and defined as pericarditis, abnormal ECG or cardiomegaly. Systolic pulmonary artery pressure of ≥40 mmHg by Doppler cardiography was regarded as pathologically raised. Muscle involvement was defined as proximal muscle weakness or serum creatinine kinase levels ≥3 times above the upper limit of normal (3.3 μkat/l). Joint involvement was defined as palpable synovitis. Renal involvement was defined as decreased glomerular filtration rate (GFR, <70% of predicted) either by [51Cr]EDTA- and iohexol clearance [11] or calculated from serum cystatin C [12] or creatinine levels.

Autoantibody analyses

Analysis for ANAs including ACAs was performed by indirect immunofluorescence using HEp-2 or HEp-20-10 cells (Euroimmun, Lübeck, Germany). Autoantibodies against

extractable nuclear antigens, including DNA topoisomerase I (ATA), SS antigens SSA and SSB, nuclear U1-RNP, Sm-antigen and Jo-1 were determined by immunoblotting (Euroimmun) at the accredited local clinical immunology laboratory (University and Regional Laboratories, Region Skåne, Lund, Sweden). ARA autoantibodies were determined by immunoprecipitation (MVZ Laboratory, Professor Seelig, Karlsruhe, Germany).

Analysis of plasma miRNAs

Plasma samples were collected in EDTA-containing tubes and stored in aliquots at −70°C. None of the plasma samples had visible haemolysis. Total RNA was purified, and a panel of miRNAs was analysed after reverse transcription using stem–loop primers, pre-amplification and quantitative RT-PCR (qRT-PCR) using specific assays from Applied Biosystems (Foster City, CA, USA). For the qRT-PCR, a microfluidic platform from Fluidigm Corp (South San Francisco, CA, USA) allowing duplicate assays for 48 miRNAs in 96 samples in one operation was used [4]. The 48 miRNA assays included 45 human and 3 Caenorhabditis elegans miRNAs (cel-miR-39, -54 and -238) (supplementary Table S1, available at Rheumatology Online). The mixture of three synthetic cel-miRNAs was spiked into the lysis buffer for use as technical normalization [4].

Data handling and statistical analyses

The demographic and paraclinical data were analysed for differences within patient groups with STATISTICA v.12 (StatSoft, Tulsa, OK, USA). Data are depicted as median (IQR). Pearson's Chi-square test was used when applicable. Differences in frequencies between groups were evaluated by Fisher's exact test. For the qRT-PCR data average, quantitation cycle (Cq) values >30 were removed from the dataset. The Cq values were then normalized using levels of spike-in controls and row means as described [4] (supplementary data, available at Rheumatology Online). The normalized miRNA Cq variables were used for autoantibody group comparisons (Kruskal–Wallis with Dunn's post-test for comparing between the four antibody groups) and in univariate comparisons (Mann–Whitney), with $P = 0.05$ as the significance threshold using GraphPadPrism v. 5.0 (GraphPad Software Inc., San Diego, CA, USA). Correction for multiple comparisons was achieved by the false discovery rate method [13]. Heat maps were generated by Genesis, release 1.7.6 [14].

For further statistical analyses, data were converted to Z-scores (mean = 0, s.d. = 1) and analysed in R, a statistical programming environment (http://www.r-project.org). All variables and groups, as well as residuals in multiple linear regression analyses, were

analysed for normal distribution and tested by the Shapiro–Wilk test. W-values >0.93 were accepted as normally distributed. Regression analyses were performed with the exclusion of data from 17 patients who were either on dialysis or had treatment other than prednisolone, leaving n = 78 patients for the analyses. The miRNA expression profiles that were associated with the two SSc disease subgroups and the four autoantibody groups (dependent variables) were used for modelling, based on logistic regression. We assessed multicollinearity (variables that do not significantly contribute further to the model fit) by variance inflation factor analysis. Variables with variance inflation factor >10 were eliminated from further analysis. Finally, the remaining explanatory variables were used to build and analyse the logistic regression models. We applied the mean difference in miRNA expression for each of the clinical phenotypes and autoantibody groups as a means of removing uninformative miRNA expression profiles from our model and of identifying interesting miRNAs to be used for our model. We included independent variables that had a P-value of 0.5 when regressed, individually, with the dependent variable (variable to be predicted) in the model, leading to a broader miRNA expression panel than the univariate analysis.

Results

Circulating miRNAs and patient set-up

For this study, a Swedish cohort of 95 patients with SSc was employed. The demographic data are depicted in Table 1. A total of 45 mature miRNAs were chosen for analysis, based on previous studies and reports of circulating miRNAs in SSc and SLE patients [4,5,15]. Based on the criterion that the miRNA should be detectable in every one of the 95 samples, 36 of these miRNAs were included in the final data analysis. The normalized miRNA expression levels were scrutinized for associations with sample age, since a possible link between miRNA expression level and sample age had been suggested in our previous study [5]. No statistically significant associations between miRNA expression level and sample age, however, were detectable in the present cohort. The normalized miRNA data were analysed for associations with SSc disease phenotype and autoantibody groups. In a second step, data analyses relating miRNA expression to organ involvement as well as logistic regression analysis were performed on 78 patients without DMARD or dialysis treatment. We reasoned that miRNA levels in DMARD-naive individuals would mirror disease-related conditions in a more straightforward way. Initial enquiries did not show any significant effect of

prednisolone on miRNA expression. Patients on concomitant prednisolone treatment were therefore admitted to the logistic regression analysis.

miRNA profiles and disease phenotype

Six miRNAs were significantly different between the lcSSc and dcSSc groups in the univariate analysis (Fig. 1). Four of these miRNAs (miR-223, -181b, -342-3p and -184) remained significant after correcting for multiple comparisons (false discovery rate <0.05) (Table 2). MiR-101, -184 and -223 were differentially expressed in analysis of both the clinical phenotype and the antibody subgroups (see below).

miRNA expression levels in SSc subgroups

The expression level of miRNAs varies between the SSc subgroups (lcSSc and dcSSc). The values of six miRNAs that differed significantly between the groups in univariate tests (Mann–Whitney U-test) are depicted for the total cohort of 95 samples. Univariate P-values are included in the figure.

Differences in plasma miRNA expression in patients with SSc (n = 95)a

Logistic regression and receiver operating characteristic (ROC) curve analyses showed an area under the curve (AUC) of 0.88 (95% CI 0.87, 0.96) for the SSc disease phenotype, indicating a good predictive power for dcSSc vs lcSSc (based on miR-29b, -101, -106a, -181b, -184, -223, -342-3p and -409-3p; supplementary Table S2).

miRNA profiles and autoantibodies

A principal components analysis based on the 36 miRNAs and the 95 samples did not reveal grouping according to the autoantibody groups (data not shown). However, by hierarchical clustering we observed grouping of more than half (19/35) of the ACA-positive patients according to their miRNA expression pattern (Fig. 2). Also, 11 miRNAs were differently expressed between the antibody groups (Table 2). Nine of these 11 miRNAs showed significant differential expression using Dunn's post-test to compare pairs of groups (Table 2Fig. 3) and 5 miRNAs (miR-409-3p, -184, -92a, -29a and -101) remained significantly differently expressed after adjusting for multiple comparisons (Table 2).

Autoantibody and miRNA levels show correlations. Heat map (unsupervised hierarchical clustering after Z-score transformation of data) of samples (horizontal axis, ACA samples are highlighted in a box) and the distribution of expression levels of 36 miRNAs (vertical axis).

Distribution of data in the four autoantibody groups (ACA (n = 35); RNP, anti-U1-RNP (n = 20); ATA, anti-DNA topoisomerase I (n = 20); and ARA, anti-RNA polymerase III antibodies) for the nine miRNAs that showed significant differences using the Kruskal–Wallis non-parametric test with Dunn's post-test (P-values for the significant comparisons are indicated on the figure).

Logistic regression and ROC curve analyses resulted in an AUC of 0.97 (95% CI 0.94, 0.99) for the ACA group (based on let-7c, miR-16, -21, -29a, -92a, -101, -184, -223 and -409-3p) and of 0.93 (95% CI 0.86, 0.99) for the ATA group (based on miR-16, -92a, -101, -155, -184 and -409-3p), suggesting high predictive power of circulating miRNA levels. AUCs were weaker for the RNP group (AUCRNP = 0.79, 95% CI 0.67, 0.90; based on miR-29a, -29c-3p, -92, 409-3p and 423-5p) and the ARA group (AUCARA = 0.68, 95% CI 0.55, 0.81; based on miR-184 and 342-3p), (supplementary Table S3, available at Rheumatology Online). In addition, reanalysis of the logistic regression calculations for all 95 patients with regard to either clinical phenotype or autoantibody group also resulted in the miRNA combinations shown above in connection with the AUC values.

miRNA and organ involvement

We tested whether organ involvement would be associated with specific miRNA profiles in DMARD-naive patients. Univariate analysis showed an association of increased miR-101 and decreased miR-92a levels with pulmonary fibrosis (P = 0.01 and 0.027, respectively). However, none of the associations remained significant after adjustment for multiple comparisons.

Discussion

Our study shows that miRNAs are differentially expressed between lcSSc and dcSSc subgroups and that the presence of specific autoantibodies is accompanied by the occurrence of particular miRNA profiles. The major findings are summarized in Fig. 4.

SSc is recognized to be a heterogeneous disease, and the notion has been put forward that SSc possibly consists of distinct disease entities [16]. In agreement with this, our results show that

the levels of specific circulating miRNAs clearly differ between the clinical phenotypes and autoantibody groups, as confirmed by a number of statistical methods. In particular, patients with ACA stand out. Half of the ACA-positive patients were grouped together in the cluster analysis. ROC analysis showed a high predictive value for ACA patients, suggesting a robust miRNA expression profile in relation to the presence of ACAs. In addition, ROC analysis showed good predictive values for patients with ATA and for the disease phenotypes. Specific miRNA profiles are therefore suggested to mirror the grouping of patients according to these classifications. Further, correlation analyses showed clear miRNA co-expression patterns beyond antibody grouping. This suggests that patients may also be linked at a molecular level through their circulating miRNA profiles in addition to their clinical phenotypes and autoantibody profiles.

MiR-409-3p expression levels are increased in ACA, compared with the other three autoantibody groups. We have previously shown that miR-409-3p levels were higher in another cohort of SSc patients (of which 41% were ACA positive) vs normal controls [5]. Thus, the combined information suggests an increase of this miR-409-3p in lcSSc. However, it is not possible to determine the absolute levels of specific miRNAs in comparison with healthy controls in the present study because healthy controls were not included. Also, there is no interstudy standardization and no standard curves included in the miRNA assays, and the row mean normalization is based on different sets of miRNAs in the two studies. No data are yet available on the biological effect of miR-409-3p with regard to pathogenesis in SSc.
MiR-184 expression levels were higher in dcSSc and in the autoantibody groups ATA and ARA. Proinflammatory cytokines such as IL-22 induce keratinocytic miR-184 expression (which in turn inhibits argonaute-2, a protein that is important for mRNA repression by miRNAs) and thus potentially lead to increased protein expression [17]. So far, however, no data can be found on miR-184 with regard to fibrosis development or angiogenesis in general and to SSc in particular.

MiR-92a levels were lower in patients with ATA and RNP compared with the ACA and ARA groups. Patients with ATA and RNP have an increased risk of developing pulmonary fibrosis compared with patients with ACA and ARA. Indeed, the presence of ACA predicts an absence of pulmonary fibrosis [18]. Interestingly, miR-92a was decreased in patients with pulmonary fibrosis. Even though the association was not significant when adjusted for multiple comparison, low miR-92a levels may be associated with pulmonary fibrosis, as our

inclusion criteria of visible fibrosis on high-resolution CT was not adjusted for the extent of fibrosis. Patients with mild fibrosis may dilute the analysis. MiR-92a is downregulated in lung tissue of patients with idiopathic pulmonary fibrosis [19]. Hypothetically, low miR-92 levels could therefore play a part in the development of pulmonary fibrosis in patients with ATA and RNP. In our previous study, miR-92a levels in plasma were decreased in SSc compared with healthy controls [5], whereas, in contrast, one other previous study presented increased miR-92a levels in sera of SSc patients [20].

A limitation of this explorative study is the cross-sectional design. Data should therefore be interpreted with some caution until future verification in validation cohorts. A second limitation is the comparatively small number of patients analysed for miRNA expression. However, the cohort is well characterized and includes analysis of 78 patients without DMARD treatment. Expression of miRNAs in this group should therefore reflect disease pathogenesis.

Conclusion

To the best of our knowledge, this is the first study to link expression levels of specific circulating plasma miRNAs to clinical phenotypes and autoantibody profiles in SSc. Circulating miRNAs may be involved in the pathogenesis and manifestations of the various SSc subtypes.

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Disclosure statement

The authors have declared no conflicts of interest.

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Table 1 Demographic characteristics of the patients with systemic sclerosis grouped according to antibody specificities[§]

	ACA	(n = 35)	**RNP**	(n = 20)	**ATA**	(n = 20)	**ARA**	(n = 20)
Female/male, no. (%)	33/2	(94/6)	18/2	(90/10)	15/5	(75/25)	16/4	(80/20)
Age at blood sampling, year	54	(45 to 63)	44	(38 to 52)	52	(33 to 65)	53	(45 to 68)
Disease duration, month, **	30	(20 to 39)	12	(7.5 to 26)	20	(12 to 51)	13	(8 to 22)
Raynauds duration, month, **	64	(27 to 128)	38	(15 to 130)	32	(12 to 63)	17	(6 to 48)
mRss, points,***	5	(3 to 9)	5	(3 to 6.5)	17	(14 to 29)	23	(13 to 31)
dcSSc/lcSSc, [c]	0/35	(0/100)	1/19	(5/95)	16/4	(80/20)	15/5	(75/25)
Esophagus	27	(77)	15	(75)	17	(85)	12	(60)
Pulmonary fibrosis, [a]	13	(37)	12	(60)	15	(75)	6	(30)
Pulmonary hypertension	3	(9)	4	(20)	4	(20)	1	(5)
Heart	11	(31)	9	(45)	7	(35)	13	(65)
Kidney	7	(20)	2	(10)	3	(15)	6	(30)
Muscle, [c]	5	(14)	10	(50)	1	(5)	2	(10)
Joint	9	(26)	6	(30)	2	(10)	3	(15)
Hemoglobin, g/L, **	135	(125 to 145)	124	(118 to 131)	125	(113 to 133)	119	(111 to 136)
Leukocytes, $x10^9$/L, ***	7.2	(5.9 to 8.8)	5	(4.1 to 5.8)	7.3	(6.3 to 9)	7.4	(6.3 to 8)
Platelets, $x10^9$/L, *	284	(228 to 331)	255	(228 to 279)	282	(257 to 352)	303	(251 to 349)
ESR, mm/h	12	(7 to 22)	22	(18 to 32)	19	(12 to 24)	17	(11 to 40)
ANA positive	35	(100)	20	(100)	20	(100)	18	(90)
Prednisolone, [a]	7	(20)	10	(50)	3	(15)	5	(25)
DMARD, [b]	1	(3)	8	(40)	3	(15)	3	(15)
Dialysis, [b]	0	(0)	0	(0)	0	(0)	4	(20)

[§] Data are shown as median (inter quartile range; IQR) or as number (percent; %). ACA = anti-centromer antibodies; ANA = anti-nuclear antibodies; ARA = anti-RNA polymerase III antibodies; ATA = anti-DNA topoisomerase 1 antibodies, dcSSc = diffuse cutaneuos SSc; DMARD

= disease modifying anti rheumatic drug; ESR = erythrocyte sedimentation rate; lcSSc = limited cutaneous SSc; IgG = immunoglobulin G; mRss = modified Rodnan skin score; Pearson Chi-square: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$; Kruskal Wallis: [*] $P < 0.05$, [**] $P < 0.01$, [***] $P < 0.001$

Table 2 Differences in plasma miRNA expression in patients with systemic sclerosis (n = 95)[§]

dcSSc vs. lcSSc	***p*-value**	**FDR** (direction of change (Fold Change))
miR-223	**0.0014**	**0.028** ↓ (0.82)
miR-181b	**0.0026**	**0.028** ↑ (1.45)
miR-342-3p	**0.0026**	**0.028** ↓ (0.59)
miR-184	**0.0031**	**0.028** ↑ (1.24)
miR-101	0.0373	0.226 ↑ (1.54)
miR-106a	0.0376	0.226 ↓ (0.86)
Autoantibody-group	***p*-value**	**FDR** (*p*-value, direction of change)
miR-409-3p	**<0.0001**	**0.0018** (<0.001, ACA↑ vs. ATA&RNP)
miR-184	**0.0006**	**0.0072** (<0.05, ACA↓ vs. ATA&ARA)
miR-92a	**0.0006**	**0.0072** (<0.05, ACA↑ vs. RNP&ATA)
miR-29a	**0.0021**	**0.0173** (<0.001, ACA↓ vs. RNP)
miR-101	**0.0024**	**0.0173** (<0.01, ACA↓ vs. ATA)
miR-21	0.0148	0.0888 (<0.05, ACA↓ vs. RNP)
miR-223	0.0229	0.0992 (<0.05, ACA↑ vs. ARA)
miR-16	0.0240	0.0992 (<0.05, ACA↑ vs. ATA)
miR-155	0.0248	0.0992 (NS)
miR-142-3p	0.0383	0.1379 (<0.05, RNP↑ vs. ARA)
let-7c	0.0439	0.1437 (NS)

[§] Data were analyzed with Mann Whitney U test (lcSSc/dcSSc) or with Kruskal-Wallis test (autoantibody groups). The *p*-values were adjusted for multiple comparisons using false-discovery rate (FRD). For the four autoantibody groups Dunn's post test was used to estimate the *p*-values in parentheses for each miRNA experiment. The miRNAs in bold represent miRNAs that display unadjusted significant differences in both the lcSSc/dcSSc and the antibody subgroups. The *p*-values in bold are significant after FDR adjustment. NS = not significant.

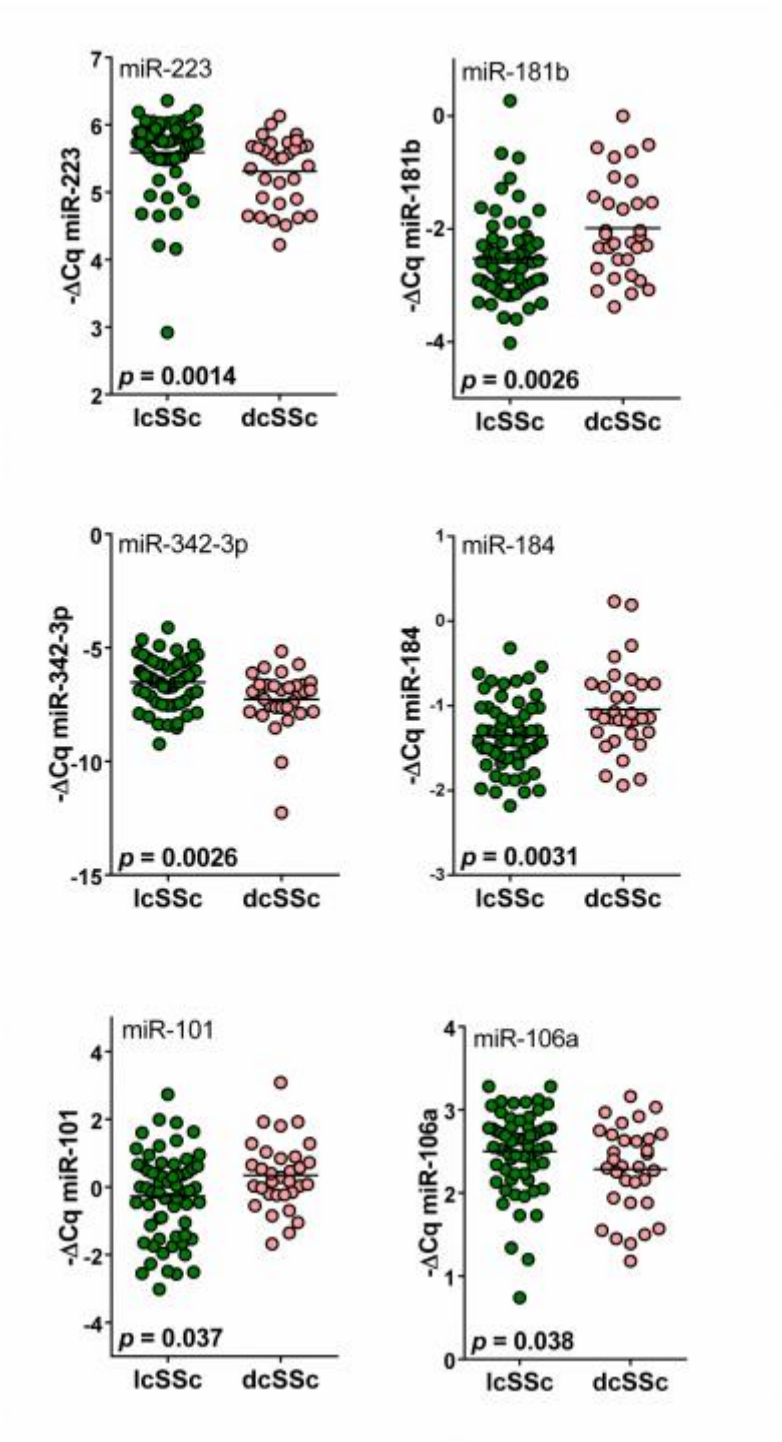

Fig. 1 MiRNA expression levels in SSc subgroups

The expression level of miRNAs varies between the SSc subgroups (lcSSc and dcSSc). The values of six miRNAs that were significantly different between the groups in univariate tests (Mann Whitney U-test) are depicted for the total cohort of 95 samples. Univariate *p*-values are included in the figure.

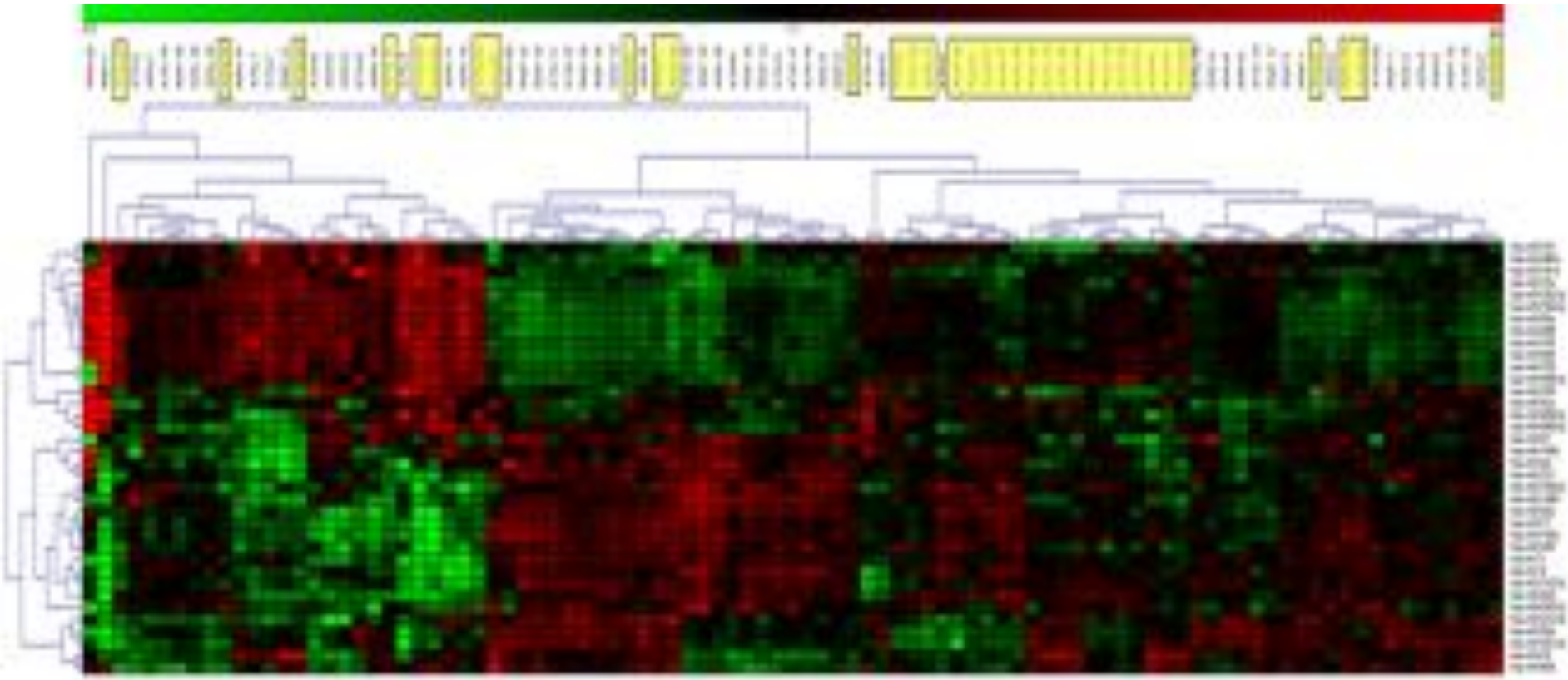

Fig. 2 Cluster analysis according to miRNA expression and autoantibody status

Autoantibody and miRNA levels show correlations. Heat map (unsupervised hierarchical clustering after Z-score transformation of data) of samples (horizontal axis, ACA samples are highlighted) and the distribution of expression levels of 36 miRNAs (vertical axis).

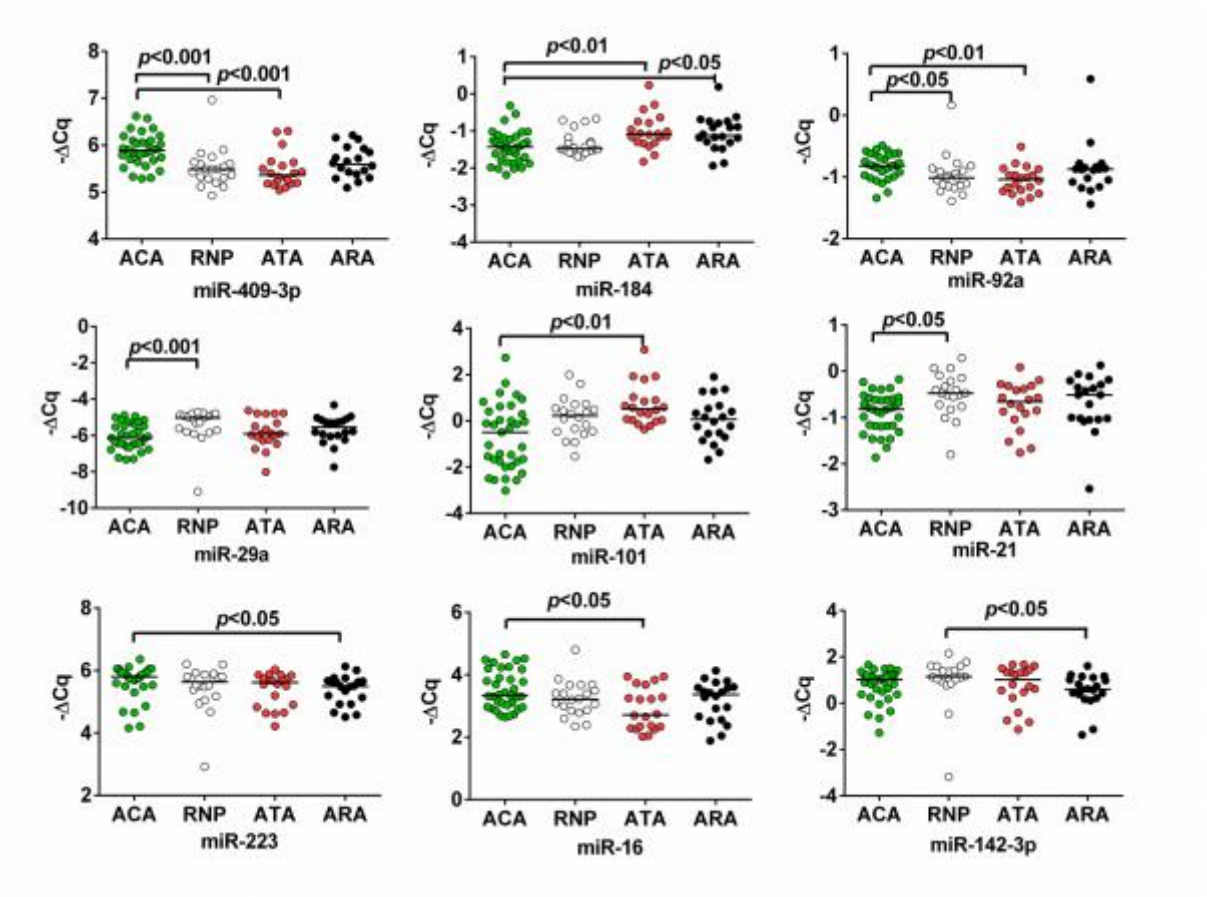

Fig. 3 MiRNA expression levels in SSc related autoantibody groups

Distribution of data in the 4 autoantibody groups (ACA, anti-centromere (n=35); RNP, anti-U1-RNP (n=20); ATA, anti-DNA topoisomerase I (n=20); and ARA, anti-RNA polymerase III antibodies) for the 9 miRNAs that showed significant differences using Kruskal-Wallis non-parametric test with Dunn's post-test (*p*-values for the significant comparisons are indicated on the figure).

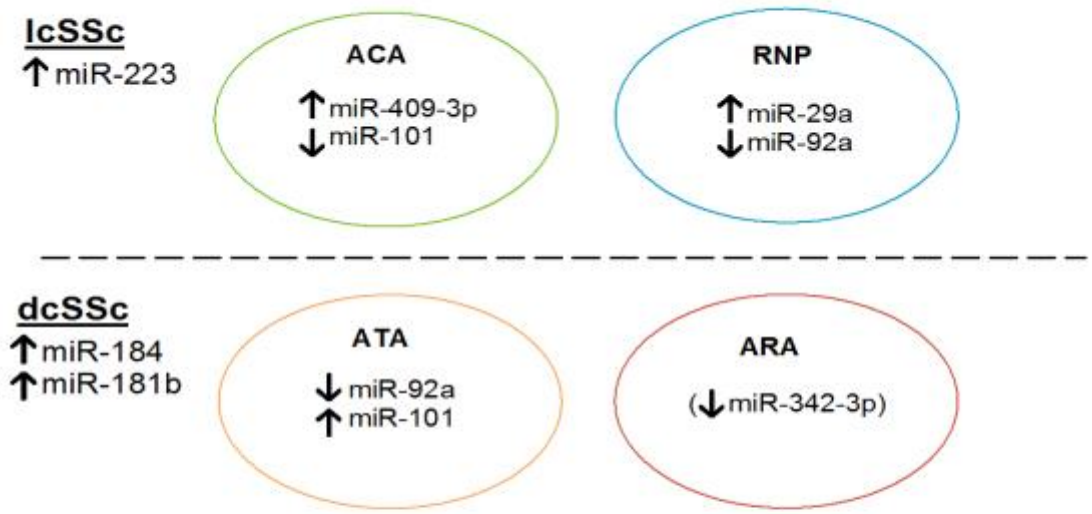

Fig. 4 Summary of plasma miRNA expression in SSc

The figure summarizes relations between microRNAs and antibody subgroups and disease phenotypes that were identified in this study.

SUPPLEMENTARY DATA 1

Supplementary Table S1. miRNAs and primers

Assay Name	Assay ID	miRBase ID	Mature miRNA sequence
hsa-let-7a-5p	000377	hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU
hsa-let-7c	000379	hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU
hsa-let-7d	002283	hsa-let-7d-5p	AGAGGUAGUAGGUUGCAUAGUU
hsa-miR-15a	000389	hsa-miR-15a	UAGCAGCACAUAAUGGUUUGUG
hsa-miR-16	000391	hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG
hsa-miR-17	002308	hsa-miR-17-5p	CAAAGUGCUUACAGUGCAGGUAG
hsa-miR-20a	000580	hsa-miR-20a	UAAAGUGCUUAUAGUGCAGGUAG
hsa-miR-21	000397	hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-24	000402	hsa-miR-24	UGGCUCAGUUCAGCAGGAACAG
hsa-miR-27a-3p	000408	hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC
hsa-miR-29a	002112	hsa-miR-29a	UAGCACCAUCUGAAAUCGGUUA
hsa-miR-29b-3p	000413	hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU
hsa-miR-29c-3p	000587	hsa-miR-29c-3p	UAGCACCAUUUGAAAUCGGUUA
hsa-miR-34a	000426	hsa-miR-34a	UGGCAGUGUCUUAGCUGGUUGU
hsa-miR-92a	000431	hsa-miR-92a	UAUUGCACUUGUCCCGGCCUGU
hsa-miR-101	002253	hsa-miR-101	UACAGUACUGUGAUAACUGAA
hsa-miR-106a	002169	hsa-miR-106a	AAAAGUGCUUACAGUGCAGGUAG
hsa-miR-122-5p	002245	hsa-miR-122-5p	UGGAGUGUGACAAUGGUGUUUG
hsa-miR-125b	000449	hsa-miR-125b	UCCCUGAGACCCUAACUUGUGA
hsa-miR-132	000457	hsa-miR-132-3p	UAACAGUCUACAGCCAUGGUCG
hsa-miR-142-3p	000464	hsa-miR-142-3p	UGUAGUGUUUCCUACUUUAUGGA
hsa-miR-145-5p	002278	hsa-miR-145-5p	GUCCAGUUUUCCCAGGAAUCCCU
hsa-miR-146a	000468	hsa-miR-146a	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-146b	001097	hsa-miR-146b-5p	UGAGAACUGAAUUCCAUAGGCU

hsa-miR-150	000473	hsa-miR-150	UCUCCCAACCCUUGUACCAGUG

Supplementary Table S1 (*continued*). miRNAs and primers

Assay Name	**Assay ID**	**miRBase ID**	**Mature miRNA sequence**
hsa-miR-154	000477	hsa-miR-154-5p	UAGGUUAUCCGUGUUGCCUUCG
hsa-miR-155	002623	hsa-miR-155	UUAAUGCUAAUCGUGAUAGGGGU
hsa-miR-199a	000498	hsa-miR-199a-5p	CCCAGUGUUCAGACUACCUGUUC
hsa-miR-181b	001098	dre-miR-181b	AACAUUCAUUGCUGUCGGUGGG
hsa-miR-184	000485	hsa-miR-184	UGGACGGAGAACUGAUAAGGGU
hsa-miR-192-5p	000491	hsa-miR-192-5p	CUGACCUAUGAAUUGACAGCC
hsa-miR-196a	247010_mat	hsa-miR-196a	UAGGUAGUUUCAUGUUGUUGGG
hsa-miR-200a	000502	hsa-miR-200a-3p	UAACACUGUCUGGUAACGAUGU
hsa-miR-200b	002251	hsa-miR-200b-3p	UAAUACUGCCUGGUAAUGAUGA
hsa-miR-203	000507	hsa-miR-203	GUGAAAUGUUUAGGACCACUAG
hsa-miR-208	000511	hsa-miR-208a	AUAAGACGAGCAAAAAGCUUGU
hsa-miR-221	000524	hsa-miR-221	AGCUACAUUGUCUGCUGGGUUUC
hsa-miR-223	002295	hsa-miR-223	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-342-3p	002260	hsa-miR-342-3p	UCUCACACAGAAAUCGCACCCGU
hsa-miR-375	000564	hsa-miR-375	UUUGUUCGUUCGGCUCGCGUGA
hsa-miR-383	000573	hsa-miR-383	AGAUCAGAAGGUGAUUGUGGCU
hsa-miR-409-3p	002332	hsa-miR-409-3p	GAAUGUUGCUCGGUGAACCCCU
hsa-miR-423-5p	002340	hsa-miR-423-5p	UGAGGGGCAGAGAGCGAGACUUU
hsa-miR-590-5p	001984	hsa-miR-590-5p	GAGCUUAUUCAUAAAAGUGCAG
hsa-miR-638	001582	hsa-miR-638	AGGGAUCGCGGGCGGGUGGCGGCCU
Cel-miR-39	000200	*Cel-miR-39*	UCACCGGGUGUAAAUCAGCUUG
Cel-miR-54	001361	cel-miR-54-3p	UACCCGUAAUCUUCAUAAUCCGAG
Cel-miR-238	000248	*Cel-miR-238*	UUUGUACUCCGAUGCCAUUCAGA

Supplementary Table S2. The logistic regression of Systemic sclerosis disease type [§]

Predictor	β	S.E. β	Wald's χ^2	*p*-value	OR	2.5% CI	97.5% CI
lcSSc vs dcSSc							
(Intercept)	-0,82	0,34	5,81	0,02	0,44	0,21	0,83
hsa.miR.29b.3p	-1,57	0,99	2,48	0,12	0,21	0,02	1,24
hsa.miR.101	-0,56	0,57	0,93	0,33	0,57	0,17	1,74
hsa.miR.106a	-0,80	0,97	0,69	0,41	0,45	0,06	2,82
hsa.miR.181b	-2,48	1,10	5,10	0,02	0,08	0,01	0,61
hsa.miR.184	-0,53	0,44	1,48	0,22	0,59	0,24	1,35
hsa.miR.223	-2,13	1,14	3,49	0,06	0,12	0,01	0,99
hsa.miR.342.3p	1,07	0,62	3,02	0,08	2,92	0,94	10,91
hsa.miR.409.3p	2,72	0,92	8,77	3,1E-03	15,20	3,25	1,3E+02

Supplementary Table S3. The logistic regression of Systemic sclerosis disease type [§]

Predictor	β	S.E. β	Wald's χ^2	*p*-value	Odds ratio	2.5% CI	97.5% CI
ACA							
(Intercept)	1,44	0,56	6,57	0,0104	4,20	1,574	15,06
hsa.let.7c	1,30	0,96	1,82	0,1774	3,67	0,621	32,28
hsa.miR.16	2,36	1,05	5,08	0,0242	10,56	1,825	128,42
hsa.miR.21	0,50	0,71	0,48	0,4868	1,64	0,401	7,17
hsa.miR.29a	-3,72	1,51	6,10	0,0135	0,02	0,001	0,30
hsa.miR.92a	0,98	0,97	1,02	0,3118	2,66	0,441	22,56
hsa.miR.101	-1,95	1,01	3,75	0,0529	0,14	0,012	0,79
hsa.miR.184	0,35	0,79	0,20	0,6565	1,42	0,316	7,43
hsa.miR.223	1,44	1,06	1,84	0,1751	4,23	0,607	45,02
hsa.miR.409.3p	3,31	1,21	7,52	0,0061	27,42	3,446	468,24
RNP							
(Intercept)	2,36	0,51	21,61	0,0000	10,59	4,539	35,13
hsa.miR.29a	1,47	1,42	1,08	0,2993	4,36	0,279	78,52
hsa.miR.29c.3p	-0,63	1,32	0,23	0,6350	0,53	0,041	7,86
hsa.miR.92a	-0,33	0,54	0,37	0,5439	0,72	0,231	2,01
hsa.miR.409.3p	-0,54	0,56	0,93	0,3361	0,58	0,177	1,70
hsa.miR.423.5p	-0,64	0,54	1,40	0,2364	0,53	0,175	1,56
ATA							
(Intercept)	3,46	0,91	14,52	0,0001	31,94	7,145	277,02
hsa.miR.16	-0,51	0,50	1,03	0,3090	0,60	0,210	1,56
hsa.miR.92a	-0,65	0,61	1,14	0,2848	0,52	0,148	1,73
hsa.miR.101	3,68	1,31	7,88	0,0050	39,50	4,525	864,44
hsa.miR.155	-3,50	1,57	4,99	0,0254	0,03	0,001	0,52
hsa.miR.184	0,51	0,54	0,89	0,3447	1,67	0,591	5,26
hsa.miR.409.3p	0,31	1,06	0,09	0,7680	1,37	0,161	12,38
ARA							
(Intercept)	1,43	0,30	22,77	0,0000	4,16	2,391	7,81
hsa.miR.184	0,27	0,29	0,90	0,3419	1,31	0,747	2,35
hsa.miR.342.3p	-0,58	0,40	2,17	0,1410	0,56	0,247	1,18