Characterisation of peripheral blood mononuclear cell microRNA in early onset psoriatic arthritis

G. Ciancio[1], M. Ferracin[2], E. Saccenti[3], V. Bagnari[1], I. Farina[1], F. Furini[1], E. Galuppi[1], B. Zagatti[3], F. Trotta[1], M. Negrini[3], M. Govoni[1]

[1]Rheumatology Unit, Department of Medical Sciences, University of Ferrara and Sant'Anna University Hospital, Ferrara, Italy; [2]Department of Experimental, Diagnostic and Specialty Medicine – DIMES, University of Bologna, Italy; [3]Department of Morphology, Surgery and Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Italy.

Abstract

Objective

To evaluate the micro-RNA (miRNA) expression profile in patients with early psoriatic arthritis (PsA) in order to assess the role of miRNAs as potential PsA biomarkers.

Methods

The expression of 723 mature miRNAs in peripheral blood mononuclear cells of early PsA patients in comparison with early-rheumatoid arthritis (ERA) patients and healthy controls (HC) was evaluated using a miRNA microarray. All patients had active disease and were naïve from treatment. The results were validated for a specific miRNA (miR-21-5p) in the entire series of patients plus an additional group of early PsA, ERA and HC using droplet digital PCR.

Results

In PsA, microarray analysis revealed a distinct pattern of 19 (vs. HC) and 48 (vs. ERA) deregulated miRNAs ($p<0.05$). The significant up-regulation of miR-21-5p both in early PsA and in ERA in comparison with HC was validated and confirmed. In PsA, miR-21-5p was found significantly down regulated after 12 weeks of therapy, which significantly correlated with the reduction of DAPSA score.

Conclusion

In early PsA, a 19- (vs. HC) and 48- (vs. ERA) miRNA signature was identified. A differential expression of miRNAs in patients with active disease makes them attractive biomarkers of psoriatic disease. MiR-21-5p was found up-regulated both in early PsA and ERA, a finding which highlights its role in the inflammatory process in general and its potential role as a therapeutic target in different inflammatory disorders. A potential role of miR-21-5p as a response to treatment biomarker in early PsA has been identified.

Key words

psoriatic arthritis, psoriasis, rheumatoid arthritis, micro-RNA, miR-21-5p, droplet digital PCR

Giovanni Ciancio, MD
Manuela Ferracin, PhD, Prof.
Elena Saccenti, PhD
Valentina Bagnari, MD
Ilaria Farina, MD
Federica Furini, MD
Elisa Galuppi, MD
Barbara Zagatti, PhD
Francesco Trotta, MD, Prof.
Massimo Negrini, PhD, Prof.
Marcello Govoni, MD, Prof.

Please address correspondence and reprint requests to:
Giovanni Ciancio,
Rheumatology Unit,
Department of Medical Sciences,
Sant'Anna University Hospital,
Via A. Moro 8,
44124 Cona, Ferrara, Italy.
E-mail: g.ciancio@ospfe.it

Received on April 5, 2016; accepted in revised form on July 11, 2016.

Funding: The authors acknowledge funding from the Regione Emilia Romagna (Early Arthritis Project) and institutional funds from the University of Ferrara.

Competing interests: none declared.

Introduction

Psoriatic arthritis (PsA) is a chronic inflammatory disorder associated with psoriasis whose pathogenesis is not yet fully understood (1). MiRNAs are small non-coding RNAs whose main function is to modulate the expression of target genes at a post-transcriptional level via translation inhibition or mRNA degradation (2). Emerging evidences have recently linked miRNA altered expression with the pathogenesis of several autoimmune inflammatory disorders, including psoriasis and a role for miRNAs as rheumatic diseases biomarkers has been hypothesised (3-5). In addition, in patients with psoriasis anti- TNF-α therapy has a relevant effect on the serum level of numerous miRNAs (6, 7).

In this study, we evaluated the global miRNA expression profile in peripheral blood mononuclear cells (PBMCs) of patients with early active and treatment naïve PsA in comparison with healthy controls (HC) and early rheumatoid arthritis (ERA) patients with the purpose to assess the role of miRNAs as potential biomarkers and their modifications linked to therapy.

Materials and methods

Patients and controls

Blood samples of 39 consecutive early PsA patients, 26 consecutive ERA patients and 16 HC were collected. To be enrolled, PsA patients had to satisfy CASPAR classification criteria (8) and ERA patients had to meet the 2010 ACR/EULAR criteria for RA classification (9).

To rule out any possible influence of drug treatment on miRNA expression, all PsA and ERA patients should have never been previously treated with glucocorticoids or disease-modifying antirheumatic drugs. Non-steroidal anti-inflammatory drugs were also suspended at least 5 days before blood sampling. During this period only pure analgesics were admitted. In PsA patients disease activity was measured by Disease Activity for Psoriatic Arthritis (DAPSA) score (10). Based on current available literature data (11, 12), DAPSA cut-off for low and high disease activity have been set at 16 and 31 points, respectively. Disease activity in ERA patients was evaluated by the Disease Activity Score (DAS28-ESR) categorised as follows: >5.1, high disease activity; ≤3.2, low disease activity; and <2.6, remission (13). Written informed consent was obtained from all the participants according to the Declaration of Helsinki and the study has been approved by the local ethics committee (Comitato Etico Unico della Provincia di Ferrara; approval code: 090586).

Sample preparation and RNA extraction

For miRNA expression studies, ten milliliters of EDTA-anticoagulated whole blood was obtained from each subject. Mononuclear leukocytes (lymphocytes and monocytes) were isolated by density gradient centrifugation as follows: EDTA-treated peripheral blood was diluted 1:2 with phosphate buffered saline (PBS) and 6–7 ml of diluted blood was laid over 3 mL of Lympholyte-H (CL5010, Cederlane laboratories Ltd) according to the manufacturer's instructions. Tubes were then centrifuged at 800 g for 20 minutes and the mononuclear cell fraction was washed once with phosphate buffered saline (PBS). RNA was isolated using Trizol LS Reagent (Invitrogen) as in manufacturer's instructions. Sample quality was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies).

MicroRNA microarray analysis

The global miRNA expression in PBMCs was investigated in the first 21 out of 39 consecutive PsA patients and in the first 10 out of 26 consecutive ERA recruited, as well as in 12 out of 16 HC using Agilent Human miRNA microarray version 2 (no. G4470B, Agilent Technologies) (14). This microarray consists of 60-mer DNA probes synthesised in situ and contains 15,000 features which represent 723 human miRNAs, sourced from the Sanger miRBASE database (Release 10.1). RNA labeling and hybridisation were performed following the manufacturer's indications. Agilent scanner and the Feature Extraction 10.5 software (Agilent Technologies) were used to obtain microarray raw-data. Microarray raw data have been submitted to ArrayExpress database (Accession number E-MTAB-3956).

Table I. Demographic, clinical and laboratory characteristics of PsA patients (n=39).

Characteristics	Value
Age, years	40.5±7.6
Male/Female, n	21/18
Disease duration, months	7.8±2.9
Psoriasis, n (%)	31 (79.4%)
Peripheral joint disease, n (%)	32 (82%)
Inflammatory low back pain, n (%)	5 (12.8%)
Established axial disease, n (%)	1 (2.5%)
Enthesitis, n (%)	25 (64.1%)
Dactylitis, n (%)	19 (48.7%)
ESR, mm/h	36±6.2
CRP, mg/dl	2.1±1.0
Positive ACPA, n (%)	0
Positive RF, n (%)	0
DAPSA	23.19±6.16

Except where indicated otherwise, values are the mean ± SD.
ESR: erythrocyte sedimentation rate (normal value: <24 mm/h); CRP: C-reactive protein (normal value: <0.6 mg/dl); ACPA: anti-citrullinated protein antibody; RF: rheumatoid factor; DAPSA: disease activity in psoriatic arthritis.

Table II. Significantly deregulated miRNAs in PBMCs from patients with PsA compared with HC.

MicroRNA	*p* (Corr)	Fold change	Regulation	HC (log2 expression)	PsA (log2 expression)
hsa-miR-21-5p	0.03	1.37	up	0.06	0.52
hsa-miR-21-3p	0.03	3.58	up	-0.69	1.14
hsa-miR-324-5p	0.03	1.65	up	0.03	0.75
hsa-miR-326	0.03	1.29	up	0.07	0.44
hsa-miR-424-5p	0.03	1.47	up	-0.16	0.39
hsa-miR-923_v12.0	0.04	2.13	up	0.38	1.48
hsa-miR-140-5p	0.05	1.22	up	0.01	0.30
hsa-miR-19b-1-5p	0.05	3.34	up	0.51	2.25
hsa-miR-301b	0.05	1.28	up	-0.08	0.27
hsa-miR-582-5p	0.05	1.44	up	-0.11	0.42
hsa-miR-106b-5p	0.05	1.20	up	0.06	0.33
hsa-miR-15a-5p	0.05	1.20	up	0.03	0.29
hsa-miR-19a-3p	0.05	1.29	up	0.05	0.42
hsa-miR-301a-3p	0.05	1.29	up	0.09	0.46
hsa-miR-627	0.05	3.17	up	0.67	2.33
hsa-miR-744-5p	0.05	2.07	up	-0.83	0.23
hsa-miR-361-3p	0.05	1.18	down	0.03	-0.21
hsa-miR-374b-5p	0.05	1.13	down	0.05	-0.12
hsa-miR-892b	0.05	4.14	down	-0.25	-2.30

PBMCs: peripheral blood mononuclear cells; PsA: psoriatic arthritis; HC: healthy controls.

Microarray results were analysed using the GeneSpring GX 13 software (Agilent Technologies). Data transformation was applied to set all the negative raw values at 1.0, followed by a quantile normalisation and a log2 transformation. Filters on gene expression were used only to keep the miRNAs expressed in at least one sample (flagged as P). Differentially expressed genes were identified by applying a moderated *t*-test in with adjusted $p<0.05$ (Benjamini and Hoechberg correction). Differentially expressed miRNAs were employed in Cluster Analysis, using the Manhattan correlation as a measure of similarity and the complete linkage rule for genes and samples clusterisation. For Cluster image generation, an additional step of normalisation on gene median across all samples was added. DIANA-miRPath v. 3.0 was used to map the unifying functional pathways of differentially expressed miRNAs (15).

Droplet digital PCR

The results were validated for a specific miRNA in the entire series of patients examined by microarray plus in other 18 PsA, 16 ERA and 4 HC, using droplet digital PCR (ddPCR), a novel and sensitive technology for miRNA quantification (16).

Reverse transcription and ddPCR

For miRNA expression validation with TaqMan assays, miR-21-5p and RNU6 were reverse-transcribed individually using TaqMan miRNA Reverse Transcription kits (Life Technologies). For each sample, 5 µl RNA was reverse-transcribed in a 15µl reaction using the standard protocol and primers specific for the two miRNAs: miR-21-5p (assay ID 000397) and RNU6 (assay ID 001973). Then, 1.3 µl of the resulting cDNA was prepared for amplification in a 20 µl reaction volume containing 10 µl 2X ddPCR Supermix for Probes (Bio-Rad) and 1 µl 20X TaqMan miRNA PCR primer probe set.

ddPCR workflow

ddPCR was performed as described (16). Each ddPCR assay mixture (20 µl) was loaded into a disposable droplet generator cartridge (Bio-Rad). Then, 70 µl of droplet generation oil for probes (Bio-Rad) was loaded into each of the eight oil wells. The cartridge was then placed inside the QX200 droplet generator (Bio-Rad). When droplet generation was completed, the droplets were transferred to a 96-well PCR plate (Eppendorf) using a Rainin multichannel pipet. The plate was heat-sealed with foil and placed in a conventional thermal cycler. Thermal cycling conditions were: 95°C for 10 min, then 40 cycles of 95°C for 15 s and 60°C for 1 min (ramping rate reduced to 2%), and a final inactivation step at 98°C for 10 min.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 6 software (GraphPad software, CA, USA). Unpaired *t*-test was used for two-group comparisons; Welch's correction was added when the variances were significantly different between the two groups. Two-sided *p*-value was always calculated. Correlation analysis between individual miRNAs and DAS28 or DAPSA scores were performed using GraphPad 6.0 software. As level of statistically significant difference a value of $p<0.05$ was assumed.

Results

Demographics and baseline data

All PsA patients (M:21, F:18; mean age: 40.5 years; mean disease duration: 7.8 months) had moderate (DAPSA ≥16 and <30) or high (DAPSA ≥31) disease activity. Main demographic, clinical and laboratory characteristics of PsA patients are detailed in Table I. All ERA patients [M:10, F:16; mean age: 45.1 years (S.D. 8.6); mean disease duration:5.5 months (S.D. 1,6)] had moderate to high active disease

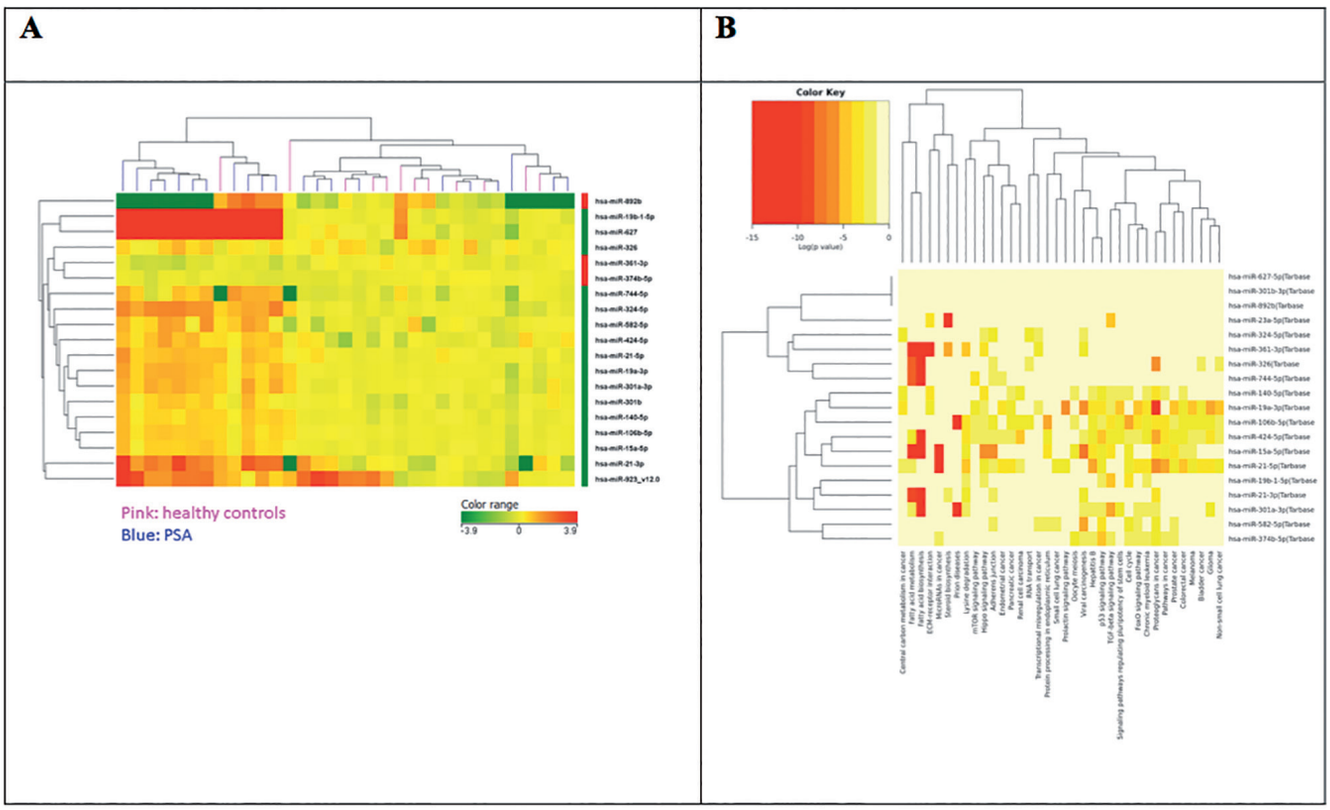

Fig. 1. MicroRNA signature of early PsA. **A**: Heatmap representation and cluster analysis 21 PsA patients (blue) and 12 healthy controls (HC, pink) performed using the list of miRNAs that are differentially expressed in PBMCs from PsA and HC (n=19). MicroRNAs that are down-regulated in PsA are coloured in green, up-regulated are coloured in red. **B**: Heatmap of differentially expressed miRNAs *vs*. significantly enriched functional pathways (Diana miRpath software). Darker colours represent higher enrichment significance at Fisher's Exact Test, as indicated by the colour bar.

[DAS28-ESR: 4.77 (S.D. 1.24)]. Rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACPA) were positive in 18 (69.2%) patients. HC were matched for age and sex [M:9; F:7; mean age: 41.5 years (S.D.3.6)]

PSA vs. HC

Microarray analysis revealed a distinct pattern of 19 significantly deregulated miRNAs in PBMCs of the 21 PsA patients compared to 12 HC ($p<0.05$) (Table II). Sixteen miRNAs were up-regulated in PsA and three were down-regulated. The hierarchical clustering based on the 19 deregulated miRNAs evidenced a good separation between PsA and HC samples (Fig. 1A). Further, we identified the validated targets (Tarbase v. 7.0 database) for the 19 differentially expressed miRNAs and their enriched functional pathways using DIANA-miRPath (15). The miRNA *vs*. pathway heatmap is provided in Fig. 1B.

PSA vs. ERA

Microarray analysis revealed a pattern of 48 miRNAs significantly deregulated in PBMCs of 21 PsA patients compared to 10 ERA (Table III). Thirteen miRNAs were up-regulated and 35 down-regulated in PsA ($p<0.05$). The hierarchical clustering based on the deregulated miRNAs evidenced a good separation between PSA and ERA samples events (Fig. 2A). Moreover, we identified the validated targets (Tarbase database) for the 48 differentially expressed miRNAs and their enriched functional pathways using DIANA-miRPath (15). The miRNA *versus* pathway heatmap is provided in Fig. 2B.

ERA vs. HC

Microarray analysis identified 114 significantly deregulated miRNAs (74 up-regulated and 40 down-regulated, adjusted $p<0.05$) in PBMCs of 10 ERA patients tested compared to 12 HC (Table IV). The hierarchical clustering based on the deregulated miRNAs evidenced a clear separation between the two groups (Fig. 3).

MicroRNA deregulated in PsA and ERA vs. HC but not in PsA vs. ERA

A restricted group of 9 micro-RNAs resulted deregulated by microarray both in PsA *vs*. HC and in ERA *vs*. HC, namely 8 up-regulated miRNAs (miR-21-5p, miR-106b-5p, miR-19a-3p, miR-19b-1-5p, miR-301a-3p, miR-301b, miR-326 and miR-424-5p) and 1 down-regulated miRNA (miR-892b) (Table II and IV). For each of these miRNAs no difference emerged from the comparison between PsA and ERA (Table III).

Validation of miR-21-5p by ddPCR

MiR-21-5p was validated in the entire series of 39 PsA patients, 26 ERA patients and 16 HC. The significant up-regulation of miR-21-5p both in PsA and in ERA patients in comparison with HC was confirmed (Fig. 4)

Correlation of miR-21-5p with disease activity in PsA and ERA

In PsA patients, no correlation between the expression of miR-21-5p and DAP-

Table III. Significantly deregulated miRNAs in PBMCs from patients with PsA compared with ERA.

MicroRNA	*p* (Corr)	Foldchange	Regulation	PsA (log2 expression)	ERA (log2 expression)
hsa-miR-224-5p	0.002	23.77	up	-1.31	3.26
hsa-miR-152	0.001	19.24	up	-0.31	3.96
hsa-miR-127-3p	0.008	18.00	up	0.05	4.22
hsa-miR-154-5p	0.003	15.59	up	-0.29	3.67
hsa-miR-575	0.009	12.17	up	0.78	4.38
hsa-miR-376a-5p	0.004	11.98	up	-1.06	2.52
hsa-miR-141-3p	0.006	11.36	up	0.87	4.38
hsa-miR-503	0.001	11.32	up	-0.04	3.46
hsa-miR-181d	0.001	10.72	up	-1.05	2.37
hsa-miR-136-5p	0.008	10.23	up	0.23	3.58
hsa-miR-381	0.009	10.00	up	0.30	3.63
hsa-miR-337-5p	0.006	9.88	up	-0.04	3.27
hsa-miR-22-5p	0.005	9.30	up	0.60	3.81
hsa-miR-133b	0.003	8.86	up	-0.89	2.26
hsa-miR-548am-5p	0.003	8.65	up	1.53	4.64
hsa-miR-421	0.005	8.61	up	-1.28	1.83
hsa-miR-382-5p	0.008	8.27	up	-1.01	2.04
hsa-miR-1	0.006	8.15	up	-0.74	2.29
hsa-miR-32-5p	0.011	7.97	up	0.56	3.56
hsa-miR-134	0.011	7.57	up	-0.07	2.85
hsa-miR-539-5p	0.005	7.56	up	-0.73	2.18
hsa-miR-299-5p	0.011	6.79	up	-1.19	1.57
hsa-miR-485-3p	0.009	6.77	up	-0.73	2.03
hsa-miR-181c-3p	0.009	6.02	up	0.61	3.20
hsa-miR-15a-3p	0.003	5.94	up	0.06	2.63
hsa-miR-629-3p	0.011	5.41	up	0.00	2.43
hsa-miR-627	0.012	5.14	up	2.33	4.70
hsa-miR-30d-3p	0.005	4.85	up	-0.51	1.77
hsa-miR-339-3p	0.006	4.72	up	-0.92	1.32
hsa-miR-335-5p	0.004	4.31	up	0.97	3.08
hsa-miR-92a-1-5p	0.012	4.26	up	-0.15	1.95
hsa-miR-598	0.006	3.58	up	0.87	2.71
hsa-miR-21-3p	0.006	2.89	up	1.14	2.68
hsa-miR-551b-3p	0.008	2.55	up	0.16	1.51
hsa-miR-877-3p	0.006	2.47	down	0.47	-0.83
hsa-miR-34a-5p	0.009	2.28	up	0.38	1.57
hsa-miR-551a	0.012	2.25	down	0.34	-0.83
hsa-miR-33b-5p	0.006	2.25	down	0.34	-0.83
hsa-miR-299-3p	0.006	2.21	down	0.32	-0.83
hsa-miR-153	0.006	2.21	down	0.31	-0.83
hsa-miR-887	0.008	2.21	down	0.31	-0.83
hsa-miR-623	0.003	2.20	down	0.31	-0.83
hsa-let-7f-1-3p	0.006	2.14	down	0.27	-0.83
hsa-miR-106a-3p	0.004	2.14	down	0.27	-0.83
hsa-miR-513a-5p	0.013	2.13	down	0.26	-0.83
hsa-miR-296-5p	0.005	2.07	down	0.22	-0.83
hsa-miR-28-3p	0.011	2.04	down	0.20	-0.83
hsa-miR-345-5p	0.006	2.02	down	0.18	-0.83

PBMCs: peripheral blood mononuclear cells; PsA: psoriatic arthritis; ERA: early rheumatoid arthritis.

SA score was found (p=0.57) (Fig. 5A). In ERA patients a trend for correlation between miR-21-5p and DAS-28 was demonstrated but it was not statistically significant (p=0.07) (Fig. 5B).

MiR-21-5p and treatment response

We investigated miR-21-5p as potential biomarker of treatment response in PsA. MiR-21-5p expression at baseline and after 12 weeks of drug therapy was assessed in 9 subjects randomly chosen among the first 21 PsA patients. Six patients were treated with methotrexate 10 mg/week+ low-dose steroids; two patients with etanercept 50 mg/week; one patient with low-dose steroids + cyclosporine 3 mg/kg/die. After 12 weeks all the 9 patients achieved a significant improvement of their disease activity (>70% reduction of DAPSA) (Fig. 6A) and a significant downregulation of miR-21-5p in eight out of nine patients was evidenced (p<0.05) (Fig. 6B).

Discussion

In this study, we have evidenced a significantly different miRNA expression profile in PBMCs of patients with PsA in comparison with healthy subjects and patients with ERA, thus proving the existence of a miRNA signature in patients with early active PsA. These findings highlight the importance of this novel class of genes as potential biomarkers in PsA and could also establish a novel starting point for understanding the pathogenetic mechanisms of this disease.

Within the miRNAs up-regulated in PsA *versus* HC, 5 have been recently described as up-regulated also in PBMCs of patients with cutaneous psoriasis in comparison with healthy controls, namely miR-21-5p, miR-21-3p, miR-15a, miR-140-5p and miR-582-5p (5). Sharing an aberrant expression of a restricted group of miRNAs is coherent with the hypothesis that psoriasis and PsA have a common pathogenic pathway (17, 18). If confirmed on a larger series of patients, this finding could also represent a starting point for future studies on targeted therapies.

A restricted group of 9 micro-RNAs resulted deregulated both in PsA *versus* HC and in ERA *versus* HC. For each of these miRNAs no difference emerged from the comparison between PsA and ERA. Among these miRNAs, miR-21-5p appeared of particular interest because of the extent of its modulation as well as its growing biological relevance. Hsa-miR-21 genetic locus generates 2 mature miRNAs, miR-21-3p (formerly, miR-21*) and miR-21-5p (formerly, miR-21) (19). Mir-21-5p was found up-regulated in malignancy (20, 21) and a role for miR-21 has recently been reported in several inflammatory and autoimmune diseases such as type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, RA, asthma, ulcerative colitis and psoriasis (22-29). Furthermore, a number of investigations suggest that miR-21 plays functional roles in many immune cells such as B cells, T cells and dendritic cells producing pathogenic cytokines involved in autoimmune and inflammatory responses (24, 30, 31). On these grounds, we focused on miR-21-5p and we confirmed its up-regulation

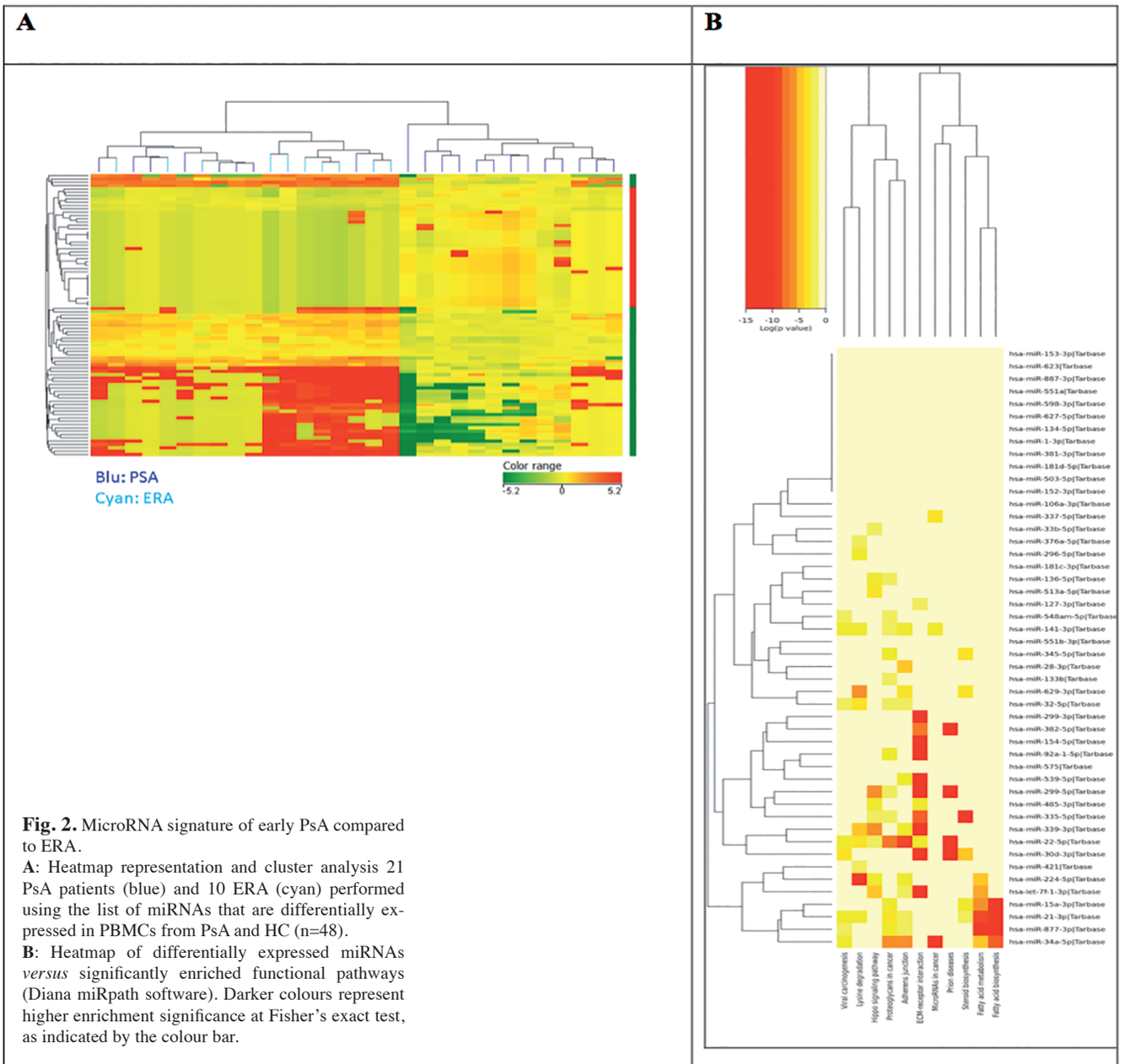

Fig. 2. MicroRNA signature of early PsA compared to ERA.
A: Heatmap representation and cluster analysis 21 PsA patients (blue) and 10 ERA (cyan) performed using the list of miRNAs that are differentially expressed in PBMCs from PsA and HC (n=48).
B: Heatmap of differentially expressed miRNAs *versus* significantly enriched functional pathways (Diana miRpath software). Darker colours represent higher enrichment significance at Fisher's exact test, as indicated by the colour bar.

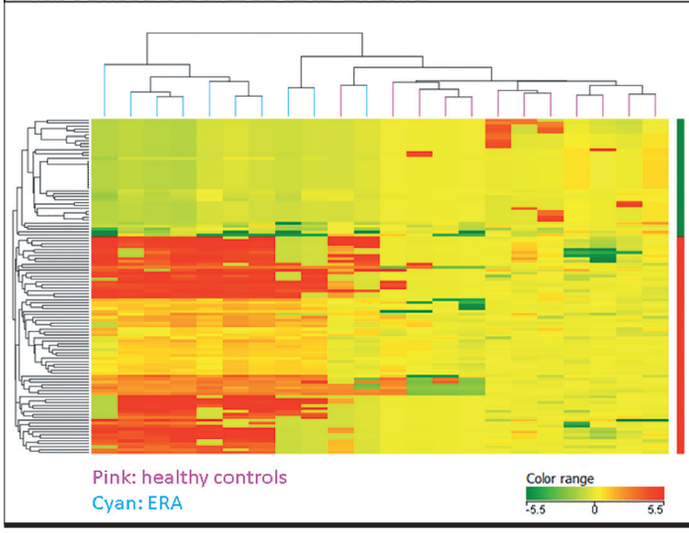

Fig. 3. MicroRNA signature of ERA. Heatmap representation and cluster analysis 10 ERA patients (cyan) and 12 HC (pink) obtained using the list of miRNAs that are differentially expressed in PBMCs from PsA and HC (n=114).

by ddPCR in the entire series of PsA and ERA patients in comparison with HC. These results seem to support the hypothesis that miR-21 could have a relevant role in the pathogenesis of various autoimmune disorders and more generally in the inflammatory process (29).
In our PsA patients the expression level of miR-21-5p did not correlate with disease activity as measured by DAPSA score, while a trend for correlation between the expression of miR-21-5p and the DAS-28 was demonstrated in ERA patients, although without statistical significance. To this regard it should be considered that DAPSA score does

Table IV. Significantly deregulated miRNAs in PBMCs from patients with ERA compared with HC.

MicroRNA	*p* (Corr)	Fold change	Regulation	HC (log2 expres.	ERA (log2 expres.	MicroRNA	*p* (Corr)	Fold change	Regulation	HC (log2 expres.	ERA (log2 expres.
hsa-miR-324-5p	<0.00000	3.25	up	0.03	1.73	hsa-miR-542-3p	0.0240	4.88	up	-0.23	2.06
hsa-miR-27a-3p	0.00002	1.73	up	0.01	0.8	hsa-miR-628-5p	0.0245	2.81	up	-0.51	0.98
hsa-miR-548am-5p	0.0001	16.01	up	0.64	4.64	hsa-miR-1	0.0251	9.33	up	-0.94	2.29
hsa-miR-503	0.0003	13.82	up	-0.32	3.46	hsa-miR-421	0.0251	4.87	up	-0.46	1.83
hsa-miR-148a-3p	0.0003	1.54	up	0.03	0.65	hsa-miR-423-3p	0.0251	3.49	up	0.03	1.84
hsa-miR-18a-5p	0.0003	1.78	up	-0.03	0.79	hsa-miR-340-5p	0.0254	3.1	up	-0.46	1.17
hsa-miR-19b-1-5p	0.0003	11.11	up	0.51	3.99	hsa-miR-30b-3p	0.0279	3.78	up	0.04	1.96
hsa-miR-34a-5p	0.0003	2.96	up	0.01	1.57	hsa-miR-502-5p	0.0321	4.16	up	0.04	2.1
hsa-miR-627	0.0003	16.3	up	0.67	4.7	hsa-miR-539-5p	0.0321	6.71	up	-0.56	2.18
hsa-miR-21-3p	0.0005	10.34	up	-0.69	2.68	hsa-miR-339-3p	0.0322	3.29	up	-0.4	1.32
hsa-miR-21-5p	0.0006	1.68	up	0.06	0.81	hsa-miR-376a-5p	0.0322	7.75	up	-0.43	2.52
hsa-miR-126-5p	0.0006	1.74	up	-0.04	0.76	hsa-miR-744-5p	0.0322	3.46	up	-0.83	0.96
hsa-miR-335-5p	0.0007	8.13	up	0.06	3.08	hsa-miR-139-5p	0.0327	6.96	up	0.74	3.54
hsa-miR-141-3p	0.0007	17.04	up	0.29	4.38	hsa-miR-493-5p	0.0327	8.76	up	0.1	3.23
hsa-miR-301b	0.0007	1.7	up	-0.08	0.68	hsa-miR-92a-1-5p	0.0423	3.53	up	0.12	1.95
hsa-miR-193a-3p	0.0008	15.72	up	0.23	4.2	hsa-miR-624-5p	0.0451	3.14	up	0.27	1.92
hsa-miR-19a-3p	0.0008	1.8	up	0.05	0.9	hsa-miR-30c-1-3p	0.0462	2.84	up	0.2	1.71
hsa-miR-152	0.0009	12.04	up	0.37	3.96	hsa-let-7f-1-3p	0.0003	1.82	down	0.03	-0.83
hsa-miR-200b-3p	0.0009	11.69	up	0.71	4.26	hsa-miR-106a-3p	0.0003	1.82	down	0.03	-0.83
hsa-miR-22-5p	0.0009	10.99	up	0.36	3.81	hsa-miR-153	0.0003	1.82	down	0.03	-0.83
hsa-miR-337-5p	0.0009	11.56	up	-0.27	3.27	hsa-miR-203	0.0003	1.82	down	0.03	-0.83
hsa-miR-379-5p	0.0009	15.15	up	0.22	4.15	hsa-miR-299-3p	0.0003	1.82	down	0.03	-0.83
hsa-miR-598	0.0009	6.59	up	-0.01	2.71	hsa-miR-551a	0.0003	1.82	down	0.03	-0.83
hsa-miR-17-3p	0.0010	1.65	up	0.05	0.78	hsa-miR-623	0.0003	1.82	down	0.03	-0.83
hsa-miR-33a-5p	0.0011	14.88	up	-0.1	3.79	hsa-miR-630	0.0003	1.82	down	0.03	-0.83
hsa-miR-542-5p	0.0014	10.73	up	-0.18	3.25	hsa-miR-877-3p	0.0003	1.82	down	0.03	-0.83
hsa-miR-136-5p	0.0014	12.9	up	-0.1	3.58	hsa-miR-887	0.0003	1.82	down	0.03	-0.83
hsa-miR-32-5p	0.0014	12.26	up	-0.06	3.56	hsa-miR-342-3p	0.0009	1.72	down	0	-0.78
hsa-miR-15a-3p	0.0020	6.47	up	-0.07	2.63	hsa-miR-342-5p	0.0012	1.71	down	0.1	-0.67
hsa-miR-181d	0.0022	5.12	up	0.02	2.37	hsa-miR-150-5p	0.0018	1.72	down	0	-0.79
hsa-miR-18b-5p	0.0023	1.63	up	0.06	0.76	hsa-miR-768-3p_v11.0	0.0022	1.56	down	0.06	-0.59
hsa-miR-301a-3p	0.0023	1.69	up	0.09	0.84	hsa-miR-1228-3p	0.0082	1.53	down	0.02	-0.6
hsa-miR-575	0.0023	15.69	up	0.41	4.38	hsa-miR-892b	0.0148	7	down	-0.25	-3.06
hsa-miR-801_v10.1	0.0026	9.52	up	0.89	4.14	hsa-miR-33b-5p	0.0165	2.04	down	0.2	-0.83
hsa-miR-106b-5p	0.0031	1.52	up	0.06	0.67	hsa-miR-1229	0.0166	2.04	down	0.2	-0.83
hsa-miR-874	0.0032	6.58	up	-0.02	2.7	hsa-miR-125b-5p	0.0166	3.89	down	0.15	-1.81
hsa-miR-154-5p	0.0033	12.5	up	0.03	3.67	hsa-miR-28-3p	0.0172	2.03	down	0.19	-0.83
hsa-miR-590-5p	0.0034	2.02	up	0.01	1.03	hsa-miR-876-5p	0.0172	2.03	down	0.19	-0.83
hsa-miR-551b-3p	0.0048	4.39	up	-0.63	1.51	hsa-miR-345-5p	0.0187	2.05	down	0.2	-0.83
hsa-miR-431-5p	0.0054	16.11	up	0.76	4.77	hsa-miR-708-5p	0.0195	2.36	down	0.36	-0.89
hsa-miR-381	0.0054	11	up	0.17	3.63	hsa-miR-130b-5p	0.0202	2.37	down	0.36	-0.89
hsa-miR-144-5p	0.0060	12.23	up	0.23	3.84	hsa-miR-628-3p	0.0236	2.42	down	0.45	-0.83
hsa-miR-130a-3p	0.0061	1.65	up	0.02	0.74	hsa-miR-193b-3p	0.0240	7.85	down	-0.39	-3.36
hsa-miR-181c-3p	0.0066	6.31	up	0.54	3.2	hsa-miR-566	0.0254	2.12	down	0.25	-0.83
hsa-miR-29b-3p	0.0069	2.19	up	0.15	1.28	hsa-miR-483-5p	0.0300	2.14	down	0.27	-0.83
hsa-miR-127-3p	0.0076	11.24	up	0.73	4.22	hsa-miR-9-5p	0.0307	2.34	down	0.39	-0.83
hsa-miR-424-5p	0.0081	1.82	up	-0.16	0.7	hsa-miR-296-5p	0.0321	2.15	down	0.28	-0.83
hsa-miR-629-5p	0.0123	4.29	up	0.22	2.33	hsa-miR-371a-5p	0.0333	2.19	down	0.3	-0.83
hsa-miR-326	0.0134	1.52	up	0.07	0.68	hsa-miR-31-5p	0.0349	2.04	down	-0.27	-1.3
hsa-miR-30e-5p	0.0135	1.56	up	0.04	0.69	hsa-miR-200a-3p	0.0369	2.17	down	0.29	-0.83
hsa-miR-758	0.0139	9.7	up	0.19	3.47	hsa-miR-513a-5p	0.0369	2.17	down	0.28	-0.83
hsa-miR-142-5p	0.0143	1.96	up	0.09	1.06	hsa-miR-760	0.0380	2.17	down	0.29	-0.83
hsa-miR-144-3p	0.0195	14.11	up	-0.16	3.66	hsa-miR-181a-2-3p	0.0390	1.59	down	-0.05	-0.73
hsa-miR-210	0.0202	2.74	up	-0.31	1.14	hsa-miR-1224-5p	0.0425	2.26	down	0.35	-0.83
hsa-miR-30d-3p	0.0209	3.48	up	-0.03	1.77	hsa-miR-514a-3p	0.0449	2.21	down	0.31	-0.83
hsa-miR-376c	0.0210	2.29	up	-0.1	1.09	hsa-miR-663a	0.0451	2.32	down	0.38	-0.83
hsa-miR-486-3p	0.0229	5.16	up	0.18	2.54	hsa-miR-557	0.0491	2.37	down	0.41	-0.83

PBMCs: peripheral blood mononuclear cells; ERA: early rheumatoid arthritis; HC: healthy controls.

not include any relevant items typical of the psoriatic disease spectrum such as enthesitis, dactylitis, axial or skin involvement that are included in the recently proposed new composite activity indices (11). Further studies carried out by applying these new activity indices would be desiderable to clarify the real role of miR-21-5P as a marker of disease activity in early PSA.

In a small sample of our PsA patients we have shown a significant deregula-

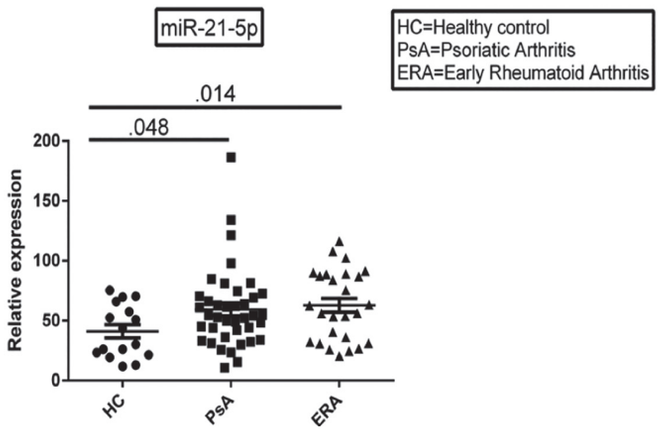

Fig. 4. Up-regulation of miR-21-5p in PsA and ERA patients. Concentration of miR-21-5p in PBMCs of 39 PsA and 26 ERA patients and 16 healthy control (HC) samples. Results are presented as miRNA copies normalised on RNU6 copies.

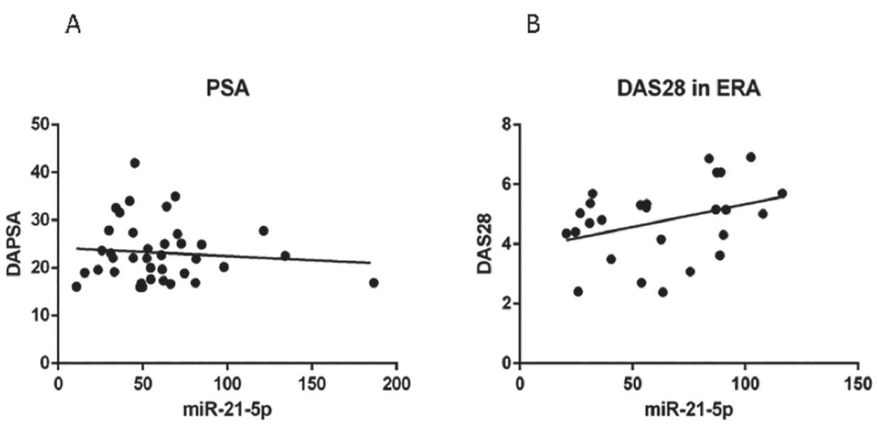

Fig. 5. Correlation of miR-21-5p with disease activity in PsA and ERA. **A**: In the 39 PsA patients, no correlation between the expression of miR-21-5p and DAPSA score was found (p=0.57). **B**: In ERA patients a trend for correlation between miR-21-5p and DAS-28 was demonstrated but it was not statistically significant (p=0.07).

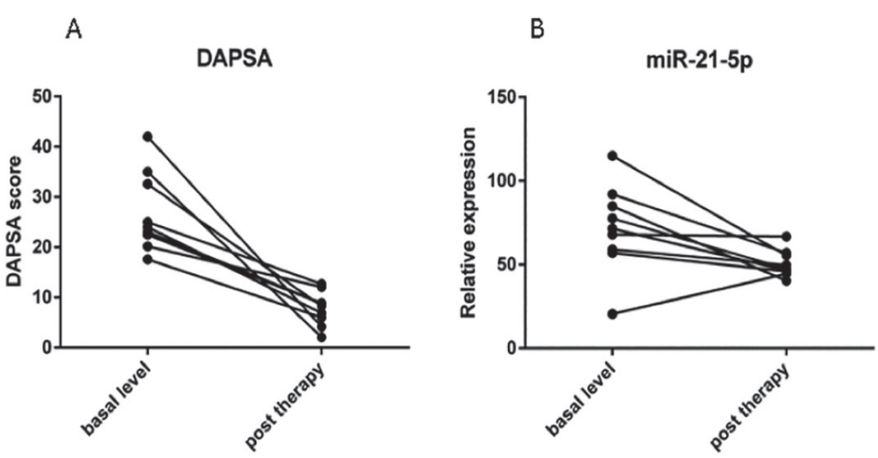

Fig. 6. DAPSA and miR-21-5p expression in PsA patients at baseline and after 12 weeks of therapy. **A**: Significant reduction of DAPSA score after 12 weeks of treatment in 9 PsA patients (p<0.0001). **B**: Significant down regulation of miR-21-5p after 12 weeks of therapy in PBMCs of 8 out 9 PsA patients (p=0.03).

tion of miR-21-5p after 12 weeks of treatment which significantly correlated with the reduction of DAPSA score. This finding justifies a further investigation of miR-21-5p as potential biomarker for treatment response in PsA and could open new scenarios in identifying a new potential therapeutic target in PsA, as indeed some recent studies seem to suggest (23, 29).

When comparing PsA and ERA, a significant deregulated miRNA expression profile was evidenced thus suggesting the existence of a different miRNA signature in patients with early active PsA also in comparison with ERA. This finding suggests that different miRNAs are specifically involved in the pathogenesis of PsA and ERA, whose specific expression profiles could be considered both as diagnostic biomarkers and new therapeutic targets.

Finally, it is noteworthy that some of the most common miRNAs that have previously been described as deregulated in PBMCs of RA, such as MiR-16, miR-146a, miR-155 and miR-223 (4, 26, 32, 33) were not confirmed as deregulated in PBMCs of our ERA patients when compared with HC. The small number of ERA patients examined with microarray could be a possible explanation. However, our ERA patients were all naive from therapy, which could call into question the previous studies on miRNAs performed on PBMC of pharmacologically treated patients. A study on larger numbers of naïve ERA patients is necessary to clarify this issue.

Some limitations of our study must be taken into account. First, the small number of patients enrolled. The rigorous inclusion criteria aimed at selecting patients free from treatment understandably limited the recruitment. Second, the lack of a comparable control group of PsA patients followed-up without treatment: we cannot rule out that the modifications of miR-21-5p observed after therapy could be the result of natural fluctuations of disease activity rather than of the treatment itself.

In conclusion, the results of this study demonstrate a miRNA signature in patients with early active PsA and the different miRNA expression profiles o PsA and ERA patients. These results could open new perspectives in the identification of diagnostic biomarkers and new therapeutic targets both in PsA and RA. A potential role of miR-21-5p in the inflammatory process in general and as a response to treatment biomarker in early PsA can be hypothesised. Further studies are warranted to confirm these results.

References

1. GLADMAN DD, ANTONI C, MEASE P, CLEGG DO, NASH P: Psoriatic arthritis: epidemiology, clinical features, course, and outcome. *Ann Rheum Dis* 2005; 64 (Suppl. 2): ii14-7.
2. FABBRI M, CROCE CM, CALIN GA: MicroRNAs. *Cancer J* 2008; 14: 1-6.
3. ALEVIZOS I, ILLEI GG: MicroRNAs as biomarkers in rheumatic diseases. *Nat Rev Rheumatol* 2010; 6: 391-8.
4. CHUROV AV, OLEINIK EK, KNIP M: MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. *Autoimmun Rev* 2015; 14: 1029-37.
5. LOVENDORF MB, ZIBERT JR, GYLDENLOVE M, ROPKE MA, SKOV L: MicroRNA-223 and miR-143 are important systemic biomarkers for disease activity in psoriasis. *J Dermatol Sci* 2014; 75: 133-9.
6. OYAMA R, JINNIN M, KAKIMOTO A *et al*.: Circulating microRNA associated with TNF-alpha signaling pathway in patients with plaque psoriasis. *J Dermatol Sci* 2011; 61: 209-11.
7. PIVARCSI A, MEISGEN F, XU N, STAHLE M, SONKOLY E: Changes in the level of serum microRNAs in patients with psoriasis after antitumour necrosis factor-alpha therapy. *Br J Dermatol* 2013; 169: 563-70.
8. TAYLOR W, GLADMAN D, HELLIWELL P, MARCHESONI A, MEASE P, MIELANTS H: Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006; 54: 2665-73.
9. ALETAHA D, NEOGI T, SILMAN AJ *et al*.: 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010; 62: 2569-81.
10. SCHOELS M, ALETAHA D, FUNOVITS J, KAVANAUGH A, BAKER D, SMOLEN JS: Application of the DAREA/DAPSA score for assessment of disease activity in psoriatic arthritis. *Ann Rheum Dis* 2010; 69: 1441-7.
11. SALAFFI F, CIAPETTI A, CAROTTI M, GASPARINI S, GUTIERREZ M: Disease activity in psoriatic arthritis: comparison of the discriminative capacity and construct validity of six composite indices in a real world. *Biomed Res Int* 2014; 2014: 528105.
12. THEANDER E, HUSMARK T, ALENIUS GM *et al*.: Early psoriatic arthritis: short symptom duration, male gender and preserved physical functioning at presentation predict favourable outcome at 5-year follow-up. Results from the Swedish Early Psoriatic Arthritis Register (SwePsA). *Ann Rheum Dis* 2014; 73: 407-13.
13. MATSUI T, KUGA Y, KANEKO A *et al*.: Disease Activity Score 28 (DAS28) using C-reactive protein underestimates disease activity and overestimates EULAR response criteria compared with DAS28 using erythrocyte sedimentation rate in a large observational cohort of rheumatoid arthritis patients in Japan. *Ann Rheum Dis* 2007; 66: 1221-6.
14. FERRACIN M, PEDRIALI M, VERONESE A *et al*.: MicroRNA profiling for the identification of cancers with unknown primary tissue-of-origin. *J Pathol* 2011; 225: 43-53.
15. VLACHOS IS, ZAGGANAS K, PARASKEVOPOULOU MD *et al*.: DIANA-miRPath v3.0: deciphering microRNA function with experimental support. *Nucleic Acids Res* 2015; 43: W460-6.
16. MIOTTO E, SACCENTI E, LUPINI L, CALLEGARI E, NEGRINI M, FERRACIN M: Quantification of circulating miRNAs by droplet digital PCR: comparison of EvaGreen- and TaqMan-based chemistries. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 2638-42.
17. CIOCON DH, KIMBALL AB: Psoriasis and psoriatic arthritis: separate or one and the same? *Br J Dermatol* 2007; 157: 850-60.
18. SCARPA R, AYALA F, CAPORASO N, OLIVIERI I: Psoriasis, psoriatic arthritis, or psoriatic disease? *J Rheumatol* 2006; 33: 210-2.
19. DOBERSTEIN K, BRETZ NP, SCHIRMER U *et al*.: miR-21-3p is a positive regulator of L1CAM in several human carcinomas. *Cancer Lett* 2014; 354: 455-66.
20. FERRACIN M, VERONESE A, NEGRINI M: Micromarkers: miRNAs in cancer diagnosis and prognosis. *Expert Rev Mol Diagn* 2010; 10: 297-308.
21. SELCUKLU SD, DONOGHUE MT, SPILLANE C: miR-21 as a key regulator of oncogenic processes. *Biochem Soc Trans* 2009; 37:918-25.
22. DAI Y, HUANG YS, TANG M *et al*.: Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. *Lupus* 2007; 16: 939-46.
23. GUINEA-VINIEGRA J, JIMENEZ M, SCHONTHALER HB *et al*.: Targeting miR-21 to treat psoriasis. *Sci Transl Med* 2014; 6: 225re1.
24. LU TX, MUNITZ A, ROTHENBERG ME: MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. *J Immunol* 2009; 182: 4994-5002.
25. MEISGEN F, XU N, WEI T *et al*.: MiR-21 is up-regulated in psoriasis and suppresses T cell apoptosis. *Exp Dermatol* 2012; 21: 312-4.
26. PAULEY KM, SATOH M, CHAN AL, BUBB MR, REEVES WH, CHAN EK: Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res Ther* 2008; 10: R101.
27. SONKOLY E, STAHLE M, PIVARCSI A: MicroRNAs: novel regulators in skin inflammation. *Clin Exp Dermatol* 2008; 33: 312-5.
28. TAKAGI T, NAITO Y, MIZUSHIMA K *et al*.: Increased expression of microRNA in the inflamed colonic mucosa of patients with active ulcerative colitis. *J Gastroenterol Hepatol* 2010; 25 (Suppl. 1): S129-33.
29. XU WD, PAN HF, LI JH, YE DQ: MicroRNA-21 with therapeutic potential in autoimmune diseases. *Expert Opin Ther Targets* 2013; 17: 659-65.
30. GARCHOW BG, BARTULOS ENCINAS O, LEUNG YT *et al*.: Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. *EMBO Mol Med* 2011; 3: 605-15.
31. SALAUN B, YAMAMOTO T, BADRAN B *et al*.: Differentiation associated regulation of microRNA expression in vivo in human $CD8^+$ T cell subsets. *J Transl Med* 2011; 9:44.
32. CHEN XM, HUANG QC, YANG SL *et al*.: Role of Micro RNAs in the Pathogenesis of Rheumatoid Arthritis: Novel Perspectives Based on Review of the Literature. *Medicine* (Baltimore) 2015; 94: e1326.
33. LI X, TIAN F, WANG F: Rheumatoid arthritis-associated microRNA-155 targets SOCS1 and upregulates TNF-alpha and IL-1beta in PBMCs. *Int J Mol Sci* 2013; 14: 23910-21.