Open Access Rheumatology: Research and Reviews

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Association of miR-145-5p, miR-143-3p and miR-146a-5p with Simplified Disease Activity Index in Rheumatoid Arthritis

Beatriz Teresita Martín Márquez[1,*], Fernanda Isadora Corona Meraz[2,*], Andrea Aguilar-Vázquez[3], Itzel Yoselin Arteaga Gallegos[4], Judith Alejandra Esparza Michel[4], Alvaro Jovanny Tovar–Cuevas[2], Milton Omar Guzmán-Ornelas[2], Roberto Carlos Rosales Gómez[2], Flavio Sandoval García[1], Oscar Pizano Martínez[1], Edy David Rubio Arellano[5], Gabriela Paola García-Ordoñez[5], Christian Juarez-Gomez[5], Mónica Vázquez-Del Mercado[1]

[1]Department of Molecular Biology and Genomics, Research Institute in Rheumatology and Musculoskeletal System (IIRSME), Health Sciences University Center (CUCS), University of Guadalajara, Guadalajara, Jalisco, Mexico; [2]Department of Biomedical Sciences, Division of Health Sciences, University Center of Tonalá (Cutonalá), University of Guadalajara, Tonalá, Jalisco, Mexico; [3]Secretariat of Sciences, Humanities and Technologies (SECIHTI), Mexico City, Mexico; [4]Department of Rheumatology, Secretariat of Sciences, Humanities and Technologies (SECIHTI), Internal Medicine Division, New Civil Hospital Dr. Juan I. Menchaca, University of Guadalajara, Guadalajara, Jalisco, México; [5]Department of Physiology, Health Sciences University Center (CUCS), University of Guadalajara, Guadalajara, Jalisco, Mexico

*These authors contributed equally to this work

Correspondence: Mónica Vázquez-Del Mercado, Department of Molecular Biology and Genomics, Research Institute in Rheumatology and Musculoskeletal System (IIRSME), Health Sciences University Center (CUCS), University of Guadalajara, Guadalajara, Jalisco, Mexico, Email dravme@hotmail.com

Purpose: MicroRNAs (miR) have emerged as key regulatory molecules in immune response and inflammation. This study investigated the association between the plasma expression levels of miR-146a-5p, miR-143-3p, miR-145-5p and rheumatoid arthritis (RA) clinical activity measured by standard tools such as the Simplified Disease Activity Index (SDAI).

Patients and Methods: Forty-eight RA patients fulfilling the EULAR/ACR 2010 criteria and 39 clinical apparently healthy subjects (HS) were included. Patients were categorized based on disease clinical activity using standard scores. Expression levels of miR were determined by RT-qPCR to be analyzed in the context of disease clinical activity, serum inflammation markers and autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP). Bioinformatic analysis was performed to assess gene interactions and signaling pathways.

Results: The expression of miR-146a-5p showed higher expression in patients with low or moderate clinical disease activity. In addition, miR-145-5p was negatively correlated with both RF and anti-CCP antibodies. Bioinformatic analysis revealed that the miRs could simultaneously regulate myosin VI (*MYO6*) and connective tissue growth factor (*CTGF*) genes.

Conclusion: Our findings suggest that the plasma levels of the analyzed miR are associated with key serological markers and low to moderate disease clinical activity in RA patients according to SDAI score. The bioinformatics data supports the potential for these miRs to regulate genes involved in RA pathology.

Keywords: RA, miR, inflammation, SDAI

Introduction

Rheumatoid arthritis (RA) is a multifaceted autoimmune disease that causes progressive joint damage accompanied by extra-articular manifestations such as interstitial lung disease, rheumatoid nodules, vasculitis, and cardiovascular complications, among others.[1] With a prevalence of approximately 0.5% to 1%, RA predominantly affects women and significantly impairs their quality of life.[2–4]

Received: 20 August 2025
Accepted: 9 December 2025
Published: 17 December 2025

RA clinical activity must be score in each outpatient visit for the best treatment decision. Therefore, clinimetry in RA for assess clinical activity, is done through some clinical tools based on the patient self-assessment and physician clinical evaluation such as: the Clinical Disease Activity Index (CDAI),[5] the Simplified Disease Activity Index (SDAI),[6] and Disease Activity Score 28 (DAS28).[7] The clinical relevance of measuring RA activity extends beyond joint health, as it is linked to quality of life and the prevalence of non-communicable diseases such as cardiovascular diseases, obesity, and diabetes mellitus. Furthermore, socioeconomic factors and universal medication access in public institutions can significantly impact life expectancy.[8–10]

The continuous search of biological, immunological and molecular markers that could be involved in the inflammation process linked to RA activity, is a fundamental task in rheumatology. Some of these markers are cytokines, chemokines and small molecules such as microRNAs (miRs).[11] Since our aim is to evaluate miR expression, the next phrases will be focused on them. The miRs are small non-coding RNA molecules, typically around 22 nucleotides in length, that regulate gene expression at the post-transcriptional level. miRs function by binding in a complementary or semi-complementary manner to messenger RNA (mRNA), which induces its degradation or translational repression.[12,13] Previous studies have demonstrated the association of miRs expression with Systemic Autoimmune Rheumatic Diseases (SARDs), given their influence on a wide range of biological processes.[12,14,15] The miRs of our interest are the miR143-3p, miR145-5p are a cluster located on human chromosome 5q32-33, transcribed as a single primary transcript that gives rise to 2 mature miRs.[16] The miR-146-5p is encoded in the human chromosome 5q33.3 producing a single transcript.[17]

Analyses conducted in RA and adjuvant-induced arthritis have highlighted the importance of miRs in susceptibility, pathogenesis, tissue damage, and treatment efficacy.[18] Specifically, miR-143-3p has been found at high levels in the plasma of patients with early RA and is overexpressed in the synovial tissue.[19,20] miR-145-5p aggravates cartilage erosion and is associated with metalloproteinase (MMP) activity in RA.[21] Furthermore, the expression of miR-146a-5p, has been shown to correlate positively with the DAS-28 score.[22,23] The meaning of 5p in the nomenclature of miRs, indicates the mature, functional strand that originates from the precursor's 5′ arm since the pre-miRNA (the hairpin precursor form) has two arms, the 5′ and the 3′.[24]

Nevertheless, a significant knowledge gap remains in comprehensively understanding the clinical applied network of theses biomarkers. While the individual contributions of miR-143-3p, miR-145-5p, and miR-146a-5p have been separately documented, no prior study has simultaneously assessed the combined expression profile of these three miRNAs and their integrated correlation with multiple indices of clinical disease activity (DAS28,[7] SDAI,[6] CDAI[5]). This integrated approach is crucial because the regulatory action of miRs is often synergistic, and their combined dysregulation may provide a robust signature for RA activity and associated pathology. Addressing this critical knowledge gap, therefore, the present study aims were to evaluate the interplay involvement of miR-143-3p, miR-145-5p, and miR-146a-5p, particularly in disease activity, and analyze their role in various molecular pathways associated with RA pathology. The potential impact of our findings on the understanding and management of RA is significant, as it could lead to the development precision medicine and tailoring therapeutic strategies.

Materials and Methods

Patients

This study included RA patients classified according to the 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) 2010 criteria.[25] Apparently clinical Healthy Subjects (HS) were recruited from the general population interested to participate. RA patients were subsequently classified according to disease activity using the DAS28, CDAI and SDAI scores, which incorporates swollen joint count, (SJT) tender joint count (TJC), patient pain assessment, and C-reactive protein (CRP) levels.[5] The research was classified as Category 1, under the General Health Law in the field of Health Research conducted in compliance with the ethical guidelines of the Declaration of Helsinki. All participants were respected and valued, as they signed an informed consent form. The protocol was approved by the Institutional Ethics Committee of the New Civil Hospital Dr. Juan I. Menchaca in Guadalajara, with file number 00047, dated May 31, 2023.

Clinical Assessment of Disease Characteristics of RA

Whole blood was collected from all subjects using the BD Vacutainer® system in EDTA tubes. The samples were centrifuged at 1500 revolutions per minute at room temperature to obtain plasma, which was stored at −80°C until use. The erythrocyte sedimentation rate (ESR) (mm/h) was determined using the Wintrobe method with the BD Sedisystem™ tube (BD Vacutainer®). The CRP was assessed using turbidimetry. Additionally, the determination of anti-CCP antibodies was assessed by enzyme-linked immunosorbent assay. To calculate the DAS28, CDAI and SDAI scores, the following parameters were added together: number of TJC and SJC based on an assessment of 28 joints, the patient global assessment (PtGA), the physician global assessment (PGA), using the visual analog scale (VAS) from 0 to 10 cm, and ESR and CRP when applied.[5] Based on the scores, RA disease activity was classified as in remission, low, moderate, or severe.

Plasma Preparation and RNA Extraction

Plasma samples were initially stored at −80°C and subsequently thawed gradually on ice before processing. For total RNA isolation, 500 µL of plasma were used. miRs were extracted using a TRIzol–chloroform protocol as described by Taylor et al.[26] Briefly, 400 µL of plasma were mixed with 1 mL TRIzol reagent, the mix incubated at room temperature for 10 min. After adding 200 µL chloroform and shaking vigorously, samples were incubated (15 min) and centrifuged at 12,000 g for 15 min at 4°C to separate phases. The upper aqueous layer (800 µL) was transferred to a fresh tube, RNA was precipitated with 1.2 mL isopropanol (added as 2×600 µL), incubated 10 min, and pelleted (12,000 g, 8 min, 4°C). Pellets were washed with 1 mL of 75% ethanol (7500 g, 5 min), air-dried (5 min), and resuspended in 30 µL nuclease-free water.

RNA Quantification and Storage

The extracted RNA samples were quantified using spectrophotometry. Only samples with a concentration greater than 100 ng/mL and acceptable quality metrics (at 1.3260/280 ratio) were selected for subsequent steps. Acceptable RNA was immediately stored at −80°C until reverse transcription.

Reverse Transcription (cDNA Synthesis)

The selected RNA samples were thawed on ice just prior to use. Reverse transcription (cDNA synthesis) was performed on 2 µL of the extracted RNA using the TaqMan Advanced cDNA Synthesis Kit (Cat. No. A28007, Applied Biosystems™) according to the recommended protocol. The resulting cDNA template was identified, aliquoted, and stored at −20°C.

Expression of miR-145-5p, miR-143-3p and miR-146a-5p

Detection and quantification of miRs were done by real-time quantitative PCR (qPCR) in triplicate from cDNA samples. Expression was normalized to miR-320a-3p, that served as the endogenous control (housekeeping) for normalization based on our previous experience.[27] The relative expression of miRs was analyzed using the comparative Ct method ($2^{-\Delta Ct}$) with the next formula ΔCT= (CT gene of interest - CT endogenous control).[28]

Bioinformatics Analysis

The databases https://www.mirbase.org/ and https://www.targetscan.org/, as well as https://www.mirnet.ca/miRNet, were used to search for genes and miR interactions.[29] In addition, each miR was analyzed individually using https://mpd.bioinf.uni-sb.de/ (miRPath V2.0) and https://dianalab.e-ce.uth.gr/ (miRPath V.3) to analyze the signaling pathways involved.

Statistical Analysis

Statistical differences for all parameters were analyzed using non-parametric tests: the Mann–Whitney *U*-test due to the data's distribution. For categorical variables, grouped differences were assessed using the Chi-Square test. Spearman correlation coefficients were calculated to determine associations between variables. Values are presented as the mean

and standard deviation (S.D). All data were analyzed using SPSS v22.0 (SPSS Inc., Chicago, IL) and GraphPad Prism v9.00 (GraphPad Software, La Jolla, CA). P <0.05 was considered statistically significant.

Results

Clinical Characteristics of RA Patients

This study included 48 RA patients and 39 HS. The RA patients were subsequently classified based on the RA disease clinical activity according to the activity scores DAS28, CDAI and SDAI. Table 1 summarizes the sociodemographic, serological markers of inflammation, clinimetry according to the scores DAS28, CDAI, SDAI for clinical activity of RA, and treatment reported across the groups.

miR-143-3p, miR-145-5p and miR-146a-5p Expression in RA is Not Different From HS

The initial analysis examined the relative plasma expression levels of miR-143-3p, miR-145-5p, and miR-146a-5p in RA patients compared to HS is shown (Figure 1). Even there is no significant difference, is interesting to note the trend of

Table 1 Clinical Assessment of Study Groups

Measurements	RA (n=48)	HS (n=53)	P value
Sociodemographic			
Female, n (%)	46(96)	38(7)	0.001
Male, n (%)	2 (4)	15(28)	0.001
Age (years)	48 (34–62)	45 (24–64)	0.126
Serum and inflammation markers levels			
ESR (mm/h)	37 ± 12	24 ± 12	0.001
CRP mg/L	13.31 ± 24.2	–	–
RF IU/mL	150 ± 203.4	–	–
Anti-CCP IU/L	200.7 ± 24.2	–	–
Disease status			
Onset* (years)	9.33 ± 8.27	–	–
Diagnosis** (years)	10.85 ± 9.32	–	–
SDAI score			
Clinical Remission, n (%)	13(27)	–	–
Low disease activity, n (%)	15(31.3)	–	–
Moderate disease activity, n (%)	15 (31.3)	–	–
High disease activity, n (%)	5 (10.4)	–	–
CDAI score			
Clinical Remission, n (%)	25 (12)	–	–
Low disease activity, n (%)	37.5 (18)	–	–
Moderate disease activity, n (%)	27.1 (13)	–	–
High disease activity, n (%)	10.4 (5)	–	–

(*Continued*)

Table 1 (Continued).

Measurements	RA (n=48)	HS (n=53)	P value
DAS28–PCR score			
Clinical Remission, n (%)	52.1 (25)	–	–
Low disease activity, n (%)	16.7 (8)	–	–
Moderate disease activity, n (%)	27.1 (13)	–	–
High disease activity, n (%)	4.2 (2)	–	–
DAS28–VSG score			
Clinical Remission, n (%)	20.8 (10)	–	–
Low disease activity, n (%)	25 (12)	–	–
Moderate disease activity, n (%)	41.7 (20)	–	–
High disease activity, n (%)	12.5 (6)	–	–
Treatment			
Methotrexate, n (%)	40 (83.3)	–	–
Sulfasalazine, n (%)	11 (29.9)	–	–
Chloroquine, n (%)	17 (35.4)	–	–
Hydroxychloroquine, n (%)	5 (10.4)	–	–
Folic acid, n (%)	38 (79.2)	–	–
Prednisone, n (%)	7 (14.6)	–	–
Leflunomide, n (%)	3 (6.3)	–	–
Rituximab, n (%)	2 (4.1)	–	–
Cholecalciferol, n (%)	14 (29.2)	–	–
No treatment, n (%)	2 (4.1)	–	–

Notes: *Time span from symptoms onset to the time of patient recruitment. **Time from diagnosis to recruitment. The results are shown in range, $\bar{x}$ ± SD, and percentages (%).
Abbreviations: RA, rheumatoid arthritis; HC, healthy controls; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide.

higher relative units (RU) of expression in the case of miR-146a-5p > miR-145-5p, being miR-143-3p the lowest expressed in HS.

miR-143-3p, miR-145-5p and miR-146a-5p Expression in RA According to Disease Progression and Symptoms Onset

When we stratified the RA patients according to this time frame: 0-≤1; 2-≤5; 6-≤10; 11-≤15; 20-≤25 and more than 25 years, we analyzed the raw data as means in each group. The miR-146a-5p showed a bimodal pattern being most expressed in early RA (0-≤1) as well as the 11- ≤15 group (Table 2). We decided to compare the RU of these miRs according to disease duration vs symptoms onset of RA patients. There was a significant correlation in miR-145-5p and miR-146a-5p (Figure 2).

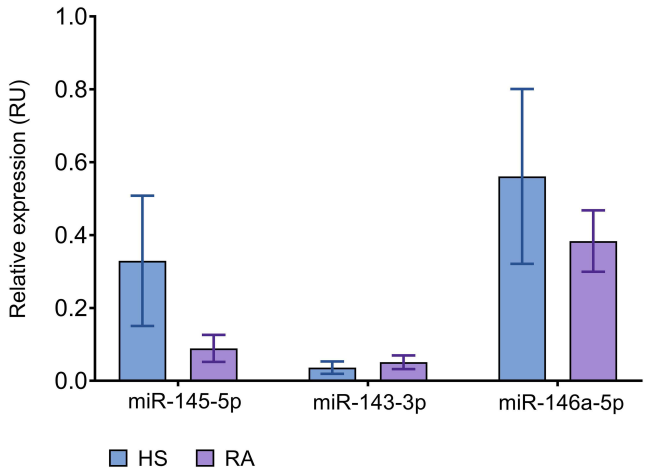

Figure 1 Relative levels of miR-145-5p, miR143-3p, and miR-146a-5p between RA patients and HS. Comparison of the relative plasma expression of miR-145, miR-143-3p, and miR-146a-5p between RA patients and HS. Data are presented as means ± SEM.
Abbreviations: miR, microRNA; RA, rheumatoid arthritis; HS, healthy subjects; RU, relative units of expression.

miR-146a-5p are Mostly Expressed in Low and Moderate Disease Activity Measured by SDAI

We investigated the relationship between the relative expression of miR-143-3p, miR-145-5p, and miR-146a-5p and the disease activity assessment tools: DAS28, CDAI and SDAI. Only SDAI score, was statistically different. We observed higher relative expression levels of miR-143-3p when comparing clinical remission versus the low clinical activity score ($P = 0.018$); clinical remission versus moderate activity ($P = 0.035$), and clinical remission versus high clinical activity

Table 2 miRs RU of Expression According to Disease Progression and Symptoms Onset

Time Frame	Disease Progression	Symptom Onset
miR-145-5p		
0 - ≤1	0.00129	0.0019
2 - ≤5	0.08176	0.0563
6 - ≤10	0.15961	0.15515
11- ≤15	0.10288	0.10288
20- ≤25	0.02665	0.03305
More ≥ 25	0.0333	0.02681
miR-143-3p		
0 - ≤1	0.0251	0.02199
2 - ≤5	0.05795	0.01382
6 - ≤10	0.06623	0.10377
11- ≤15	0.05661	0.05012
20- ≤25	0.00158	—
More ≥ 25	0.00583	0.00335

(*Continued*)

Table 2 (Continued).

Time Frame	Disease Progression	Symptom Onset
miR-146a-5p		
0 - ≤1	0.58271	0.57614
2 - ≤5	0.30484	0.44165
6 - ≤10	0.36457	0.33786
11- ≤15	0.5597	0.55956
20- ≤25	0.08521	0.12774
More ≥ 25	0.134	0.06842

Notes: miR-145-5p, miR-143-3p, and miR-146a-5p stratified by disease progression and symptom onset time frame. The results are shown as means. Relative units (RU) of expression.

(P = 0.030). Similarly, miR-146a-5p showed significantly higher differential expression when comparing the clinical remission with low clinical activity (P = 0.006) and the clinical remission versus moderate activity (P = 0.019). In contrast, the relative expression of miR-145-5p did not show a statistically significant association (Figure 3).

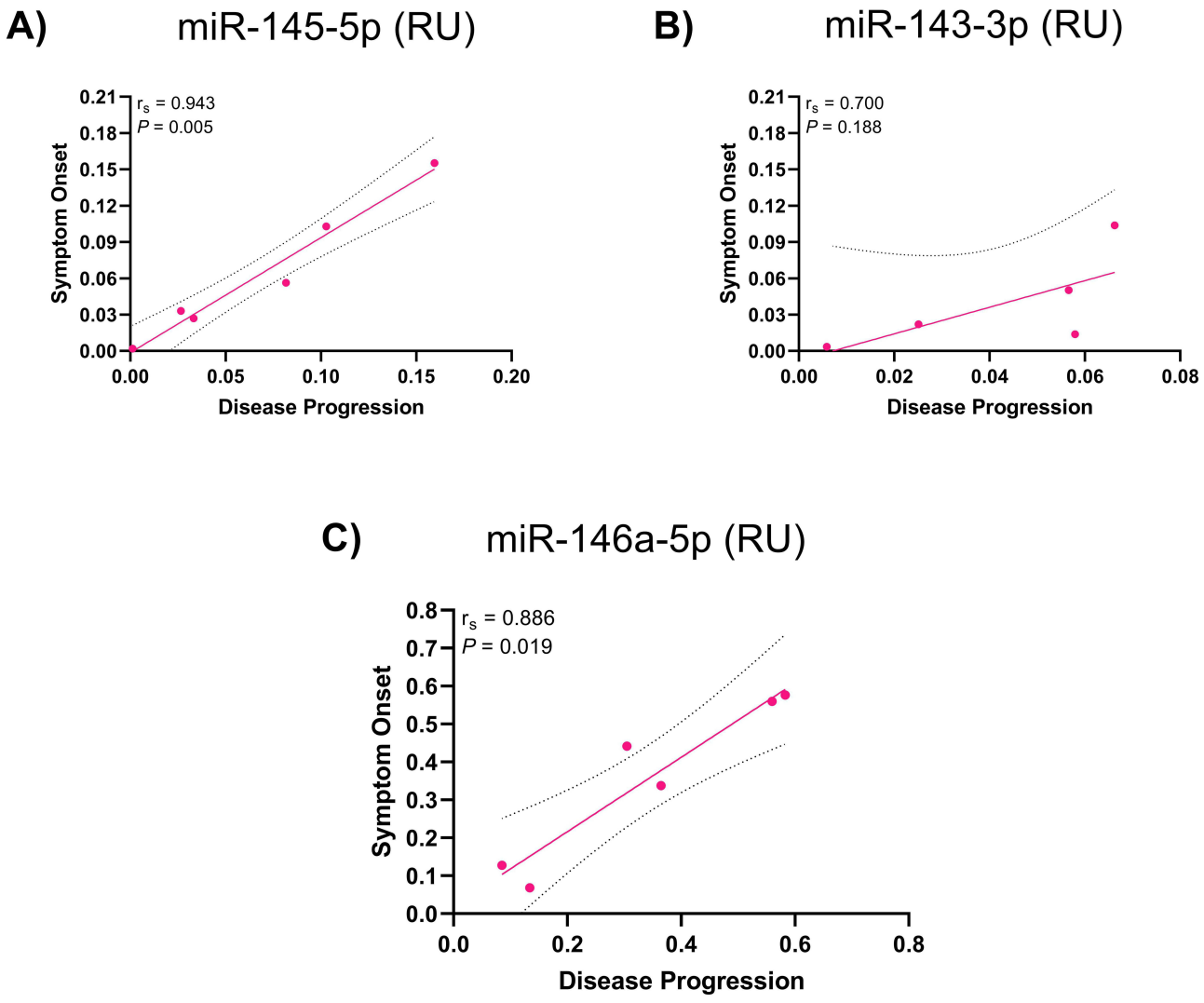

Figure 2 Correlation between disease progression and symptom onset based on miRs expression data: miR-145-5p (**A**), miR143-3p (**B**), and miR-146a-5p (**C**).

https://doi.org/10.2147/OARRR.S561973

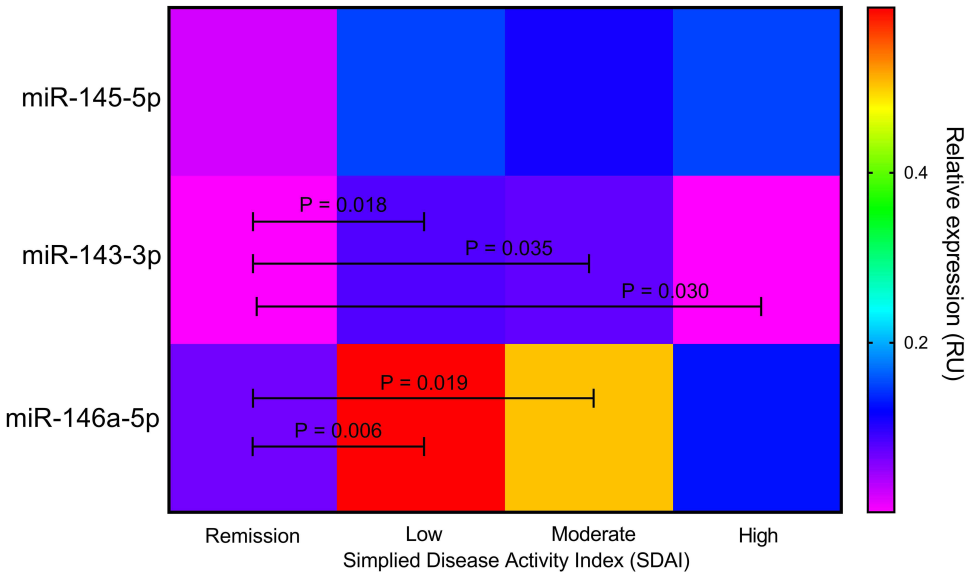

Figure 3 Relative levels of miR-145-5p, miR143-3p, and miR-146a-5p in RA patients according to SDAI score. The heat map illustrates the differences in the relative expression of miR-145-5p, miR-143-3p, and miR-146a-5p according to SDAI score in RA patients.
Abbreviations: miR, micro-RNA; RA, rheumatoid arthritis; SDAI, simplified disease activity index; RU, relative expression units.

Correlation of miR-143-3p, miR-145-5p and miR-146a-5p with Titers of RF and Anti-CCP Antibodies

Following the analysis of the association between the studied miRs and SDAI score, we conducted a correlation analysis involving their relative expression levels, acute-phase reactants, Rheumatoid Factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibodies. We found significant positive correlations between the relative expression of miR-143-3p and RF *(rs* = 0.409, *P* = 0.015), and between miR-146a-5p and RF *(rs* = 0.341, *P* = 0.036).Conversely, we observed a strong negative correlation between the relative expression levels of miR-145-5p and anti-CCP *(rs* = −0.745, *P* = 0.000254). These findings are illustrated in Figure 4.

Bioinformatic Analysis of the Evaluated miRs

To process and analyze the biological data related to the studied miRs, we performed a bioinformatic analysis to identify relevant target genes and signaling pathways in RA. The results showed that miR-143-3p, miR-145-5p, and miR-146a-5p could simultaneously regulate the genes for Myosin VI (*MYO6*) and Connective Tissue Growth Factor (*CTGF*), along

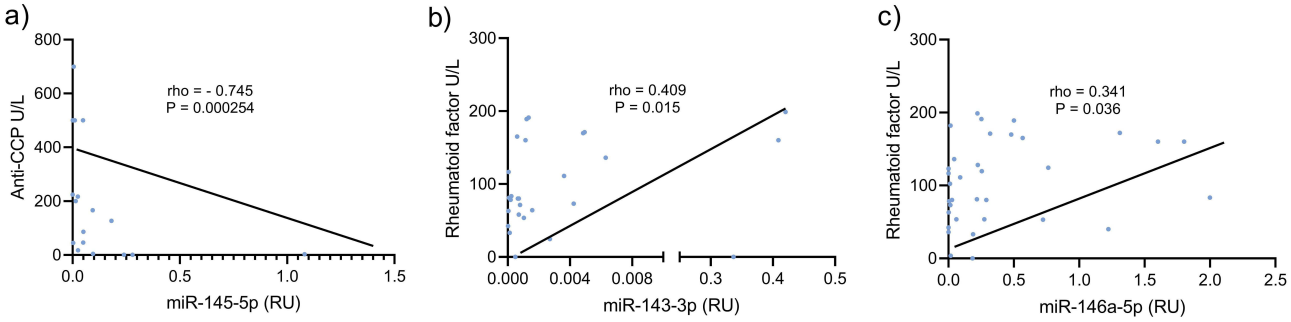

Figure 4 Correlation of miRs with RF and anti-CCP antibodies: miR-145-5p (**a**), miR143-3p (**b**), and miR-146a-5p (**c**). (**a**) miR-145-5p negatively correlates with anti-CPP levels; (**b**) miR-143-3p positively correlates with RF; (**c**) miR-146a-5p correlates positively with RF.

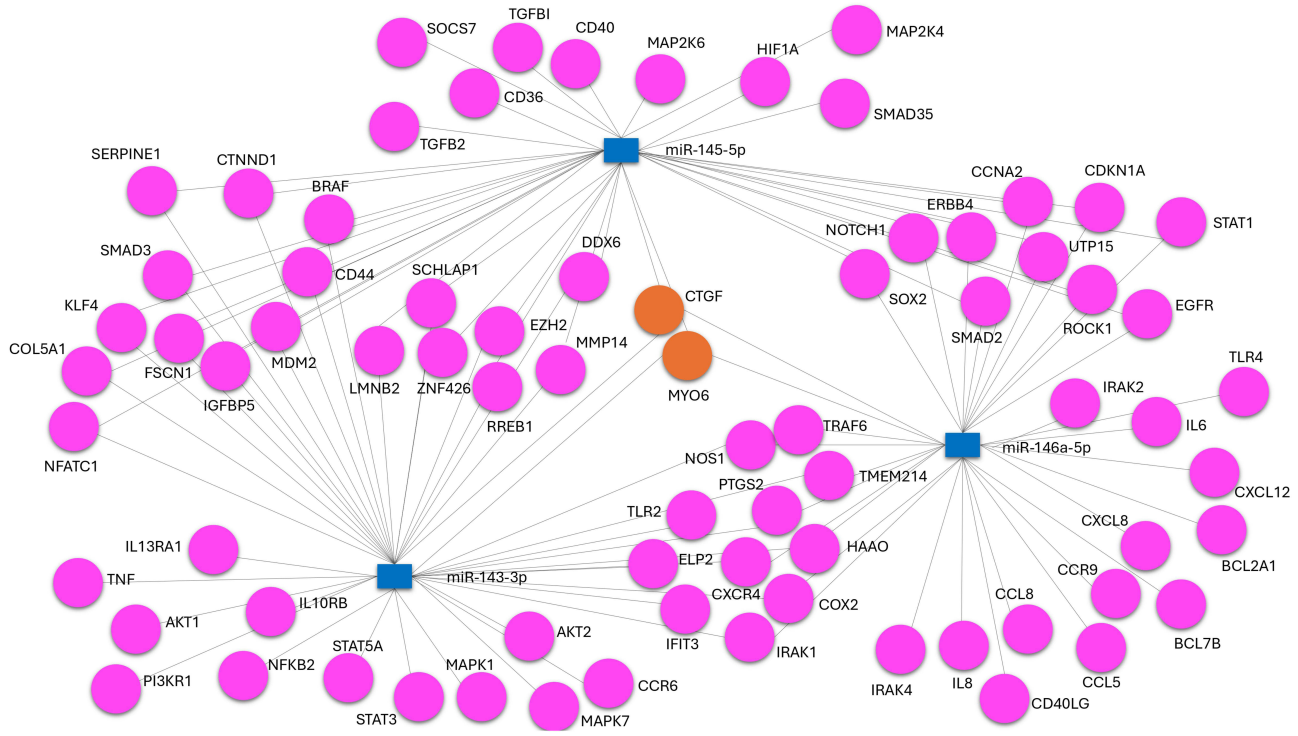

Figure 5 Genes co-regulated by miRs miR143-3p, miRs-145-5p and miR-146a-5p. Bioinformatic analysis performed using miRnet on March 19, 2025. The Orange circles indicate genes co-regulated by the three miRs analyzed (miR143-3p, miRs-145-5p, and miR-146a-5p), and the pink circles indicate genes co-regulated by two miRs.

with multiple genes related to inflammation and immune response. This potential co-regulation highlights a novel molecular mechanism underlying RA pathogenesis. Individually, the miRs were also observed to regulate genes associated with inflammation, antibody production, cell response, and cytokine production (Figure 5 and Table 3).

Table 3 Pathways Associated with the Evaluated miRs

miRs	Pathways*	Hits (P)
miR-143-3p	Bone trabecula formation	0.041
	Cells and molecules involved in local acute inflammatory response	0.014
	ApoE and miR-146 in inflammation and atherosclerosis	0.012
	Negative regulation of cytokine secretion involved in immune response	0.014
	Cytokine secretion involved in immune response	0.017
	Immune response-regulating cell surface receptor signaling pathway	0.049
miR-145-5p	Inflammatory response	0.037
	Regulation of immune system process	4.04e-6
	Positive regulation of immune system process	1.38e-5
	Immune system process	3.00e-4
	Immune system development	0.001

(*Continued*)

https://doi.org/10.2147/OARRR.S561973

Table 3 (Continued).

miRs	Pathways*	Hits (P)
miR-146a-5p	Regulation of immune response	0.006
	B cell activation involved in immune response	0.014
	Immune response-regulating signaling pathway	0.010
	Positive regulation of immune response	0.014
	Positive regulation of innate immune response	0.015
	Regulation of innate immune response	0.019
	Immunoglobulin production involved in immunoglobulin mediated immune response	0.023
	Somatic diversification of immunoglobulins	0.028
	Cytokine-mediated signaling pathway	1.67e-15
	Response to cytokine	4.51e-15
	Cellular response to cytokine stimulus	6.63e-16
	TLR Signaling pathway	4.99e-11
	Cytokine Signaling in Immune system	1.55e-10
	Immune response	1.88e-10
	Immune system process	1.88e-10
	Immune system	5.65e-10
	Innate immune response	1.65e-9
	Inflammatory response	8.44e-6
	Inflammatory cell apoptotic process	0.010

Notes: *https://mpd.bioinf.uni-sb.de/mirna.html?mirna=hsa-miR-143-3p&organism=hs (analysis conducted on March 18, 2025).
Abbreviations: has, Homo sapiens; ApoE, apolipoprotein E; TLR, toll-like receptor.

Discussion

Among the epigenetic factors crucial to RA pathophysiology, miRs regulate gene expression by targeting miRs for degradation, thus influencing cellular responses and disease pathogenesis. miRs hold the potential to serve as biomarkers for patient stratification, monitoring disease activity, and clinical treatment guides.[14,30]

miRs are promising candidates to be proved the clinical utility for the diagnosis in RA. Equally important is the putative role of some miRs in monitoring disease activity as our results showed with the miR-146a-5p, guiding comprehensive patient follow-up.[31] Even though there is no significant difference between RA and HS in the expression of the miRs studied in this work, it is interesting to note the trend of higher relative units (RU) of expression in the case of miR-146a-5p > miR-145-5p, with miR-143-3p being the lowest expressed in HS, reminding the importance of these miRs in the proinflammatory response inhibition, especially related to miR-143-3p.[20]

A key objective of our study was to investigate the association between plasma expression levels of miR-143-3p, miR-145-5p, miR-146a-5p and disease activity, assessed by SDAI score in RA patients. Our analysis of miR-143-3p revealed a non-linear association with disease activity. Its relative expression was lowest during the remission phase but showed significant differences when comparing remission with the low activity, moderate activity, and high activity phases. Specifically, the expression of miR-143-3p increased in the low and moderate activity phases compared to remission but subsequently decreased in the high activity phase. This dynamic suggests that miR-143-3p, may reflect a specific stage of the immune response. Yue et al have demonstrated a significantly higher relative expression of miR-

143-3p in the plasma levels of RA patients with erosions, highlighting its potential as a promising predictor of bone erosion progression in this disease.[32] On the other hand, Shen et al found elevated levels of miR-143-3p in CD4+ T cells from patients with moderate RA compared to those with severe disease.[33]

Furthermore, it has been reported in a collagen-induced arthritis (CIA) animal model that miR-143-3p inhibition is associated with a decrease in proinflammatory cytokines, such as IL-1β and IL-6, suggesting a potential therapeutic target for human RA.[33] On the other hand, low expression of miR-143-3p may be related to chronic inflammatory pain in RA, as it was found in a CIA model to negatively regulate pain-associated target genes, including MAS-related GPR family member E (MRGPRE), prostaglandin-endoperoxide synthase 2 (PTGS2), and tumor necrosis factor alpha (TNF-α).[34] Furthermore, this miR also influences cell proliferation and apoptosis by acting on insulin-like growth factor 1 receptor (IGF1R) and insulin-like growth factor binding protein 5 (IGFBP5), regulating the Ras/p38 mitogen-activated protein kinase (MAPK) signaling pathway, which is crucial in RA.[20] In addition, miR-145-5p and miR-143-3p are known to form a cluster that plays a key role in regulating cell differentiation and proliferation, modulating inflammation, and participating in the response to fibrosis and tissue damage.[35]

Notwithstanding, miR-146a-5p increased only in low and moderate disease activity, early RA (0–1 year) and 11–15 years of disease duration and symptom onset. This bimodal pattern of miR-146a-5p, could behave as a homeostatic mechanism that is activated to dampen inflammation by regulating toll like receptor (TLR) and cytokine signaling targeting tumor necrosis factor receptor-associated factor 6 (TRAF-6) and interleukin-1 receptor-associated kinase 1 (IRAK-1). This regulatory function was supported by a study by Tavasolian et al, who highlighted miR-146a-5p as one of the most consistently upregulated miR in CD4+ T cells of RA synovial tissue.[36] The utility of miR-146a-5p as a systemic biomarker in diverse populations was further confirmed by El-Bakry et al, who evaluated its circulating levels in an Egyptian RA cohort.[37]

For their part, Mookherje et al conducted one of the first analyses on whole blood to determine the relative expression of miR-146a-5p in patients with RA reporting a significant linear correlation between the expression of miR-146a-5p in peripheral blood mononuclear cells (PBMCs) and whole blood in RA.[22] In addition, Bae et al performed a comprehensive meta-analysis to evaluate the relationship between the relative expression of miR-146a-5p, disease activity (DAS-28) and ESR.[38] Moreover, recent network analyses by Chen et al identified miR-146a-5p as a potential immune effector molecule associated with RA through its linkage with genes like lymphocyte cell-specific protein-tyrosine kinase (LCK) and forkhead box C1 (FOXC1).[39] These findings reinforce the role of miR-146a-5p in the immunopathology of RA.

Our bioinformatic analysis of biological data related to the miRs studied has revealed a potentially remarkable breakthrough. We discovered that miR-143-3p, miR-145-5p, and miR-146a-5p can simultaneously regulate myosin VI (*MYO6*) and connective tissue growth factor (*CTGF*) genes, as well as multiple genes related to inflammation and immune response. This potential co-regulation reveals a new molecular mechanism underlying the pathogenesis of RA, a finding that could have a significant impact on future studies in this field.

The finding of MYO6 as a common regulated gene is significant, as its activity is related to the regulation of actin dynamics, and its deficiency limits joint inflammation and bone destruction in arthritic mice. Furthermore, Tan et al, observed that MYO6 deficiency limits joint inflammation and bone destruction in arthritic mice. In addition, they observed increased MYO6 expression in CD14+ monocytes in RA patients compared to HS.[40] The simultaneous regulation of CTGF, a known profibrotic cytokine involved in cartilage and bone destruction, suggests that the miRs-143-3p/145-5p/146a-5p cluster acts synergistically to influence both inflammatory and tissue remodeling processes.

Study Limitations

The main limitation is the relatively small sample size (n = 48 RA patients), which restricts statistical power and requires confirmation of the differential expression patterns observed in larger independent populations. Furthermore, the cross-sectional design precludes establishing causality, making it difficult to determine whether miRs are a cause or a consequence of fluctuations in disease activity over time. This underscores the need for further research to elucidate this relationship. Finally, because we measured expression in plasma, we cannot accurately determine the cellular origin of these circulating miRs or fully account for potential confounding factors, such as specific drug regimens, that could

influence their systemic levels, further highlighting the need for further research to address these limitations and deepen our understanding of miRs and RA.

Conclusions

In conclusion, our study demonstrates that circulating levels of the miR-143-3p/miR-145-5p were the lowest expressed in RA. In contrast, the miR-146a-5p presented a bimodal peak of expression according to disease duration and symptom onset. In addition, this miR-146-5p was mostly expressed in low to moderate clinical activity in RA assessed by SDAI score. Finally, our bioinformatic analysis highlights a novel mechanism involving the potential co-regulation of *MYO6* and *CTGF* by all three miRs, linking the cluster to both inflammatory processes and tissue remodeling in RA pathogenesis.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available in the FigShare repository available at: dx.doi.org/10.6084/m9.figshare.30596270.

Ethical Approval

This study was approved by the Institutional Ethics Committee of the Nuevo Hospital Civil "Dr. Juan I. Menchaca" in Guadalajara, Jalisco, Mexico, under file number 00047, dated May 31, 2023. All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and the General Health Law in the field of Health Research (Category I).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The authors received no specific funding for this work.

Disclosure

The author(s) report no conflicts of interest in this work.

References

1. Chauhan K, Jandu JS, Brent LH, Al-Dhahir MA. Rheumatoid Arthritis. *StatPearls*. 2025.
2. Jiang Y, Zhong S, He S, et al. Biomarkers (mRNAs and non-coding RNAs) for the diagnosis and prognosis of rheumatoid arthritis. *Front Immunol*. 2023;14:1087925. doi:10.3389/fimmu.2023.1087925
3. Venetsanopoulou AI, Alamanos Y, Voulgari PV, Drosos AA. Epidemiology of rheumatoid arthritis: genetic and environmental influences. *Expert Rev Clin Immunol*. 2022;18(9):923–931. doi:10.1080/1744666X.2022.2106970
4. Deane KD, Demoruelle MK, Kelmenson LB, Kuhn KA, Norris JM, Holers VM. Genetic and environmental risk factors for rheumatoid arthritis. *Best Pract Res Clin Rheumatol*. 2017;31(1):3–18. doi:10.1016/j.berh.2017.08.003
5. Smolen JS, Aletaha D. Scores for all seasons: SDAI and CDAI. *Clin Exp Rheumatol*. 2014;32(5 Suppl 85):S–75–9.
6. Aletaha D, Smolen J. The simplified disease activity index (SDAI) and the clinical disease activity index (CDAI): a review of their usefulness and validity in rheumatoid arthritis. *Clin Exp Rheumatol*. 2005;23(5 Suppl 39):S100–8.
7. Wells G, Becker JC, Teng J, et al. Validation of the 28-joint disease activity score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis*. 2009;68(6):954–960. doi:10.1136/ard.2007.084459
8. George MD, Baker JF. The obesity epidemic and consequences for rheumatoid arthritis care. *Curr Rheumatol Rep*. 2016;18(1):6. doi:10.1007/s11926-015-0550-z
9. Song Y, Chen Y, Wen L, et al. Health-related quality of life profiles in patients with rheumatoid arthritis: a latent profile analysis. *Front Public Health*. 2024;12:1478376. doi:10.3389/fpubh.2024.1478376

10. Vazquez-Del Mercado M, Gomez-Banuelos E, Chavarria-Avila E, et al. Disease duration of rheumatoid arthritis is a predictor of vascular stiffness: a cross-sectional study in patients without known cardiovascular comorbidities: a STROBE-compliant article. *Medicine*. 2017;96(33):e7862. doi:10.1097/MD.0000000000007862
11. Seyedi D, Espandar N, Hojatizadeh M, Mohammadi Y, Sadri F, Rezaei Z. Noncoding RNAs in rheumatoid arthritis: modulators of the NF-kappaB signaling pathway and therapeutic implications. *Front Immunol*. 2024;15:1486476. doi:10.3389/fimmu.2024.1486476
12. Chang C, Xu L, Zhang R, et al. MicroRNA-mediated epigenetic regulation of rheumatoid arthritis susceptibility and pathogenesis. *Front Immunol*. 2022;13:838884. doi:10.3389/fimmu.2022.838884
13. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol*. 2018;9:402. doi:10.3389/fendo.2018.00402
14. Ceribelli A, Satoh M, Chan EK. MicroRNAs and autoimmunity. *Curr Opin Immunol*. 2012;24(6):686–691. doi:10.1016/j.coi.2012.07.011
15. Smolarz B, Durczynski A, Romanowicz H, Szyllo K, Hogendorf P. miRNAs in cancer (Review of Literature). *Int J Mol Sci*. 2022;23(5). doi:10.3390/ijms23052805
16. Cordes KR, Sheehy NT, White MP, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 2009;460(7256):705–710. doi:10.1038/nature08195
17. Paterson MR, Kriegel AJ. MiR-146a/b: a family with shared seeds and different roots. *Physiol Genomics*. 2017;49(4):243–252. doi:10.1152/physiolgenomics.00133.2016
18. Peng X, Wang Q, Li W, et al. Comprehensive overview of microRNA function in rheumatoid arthritis. *Bone Res*. 2023;11(1):8. doi:10.1038/s41413-023-00244-1
19. Auer ED, de Carvalho Santos D, de Lima IJV, Boldt ABW. Non-coding RNA network associated with obesity and rheumatoid arthritis. *Immunobiology*. 2022;227(6):152281. doi:10.1016/j.imbio.2022.152281
20. Yang Z, Wang J, Pan Z, Zhang Y. miR-143-3p regulates cell proliferation and apoptosis by targeting IGF1R and IGFBP5 and regulating the Ras/p38 MAPK signaling pathway in rheumatoid arthritis. *Exp Ther Med*. 2018;15(4):3781–3790. doi:10.3892/etm.2018.5907
21. Wang X, Tang K, Wang Y, et al. Elevated microRNA-145-5p increases matrix metalloproteinase-9 by activating the nuclear factor-kappaB pathway in rheumatoid arthritis. *Mol Med Rep*. 2019;20(3):2703–2711. doi:10.3892/mmr.2019.10499
22. Mookherjee N, El-Gabalawy HS. High degree of correlation between whole blood and PBMC expression levels of miR-155 and miR-146a in healthy controls and rheumatoid arthritis patients. *J Immunol Methods*. 2013;400-401:106–110. doi:10.1016/j.jim.2013.10.001
23. Abou-Zeid A, Saad M, Soliman E. MicroRNA 146a expression in rheumatoid arthritis: association with tumor necrosis factor-alpha and disease activity. *Genet Test Mol Biomarkers*. 2011;15(11):807–812. doi:10.1089/gtmb.2011.0026
24. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res*. 2019;47(D1):D155–D162. doi:10.1093/nar/gky1141
25. Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. *Rheumatology*. 2012;51(suppl_6):vi5–vi9. doi:10.1093/rheumatology/kes279
26. Taylor CJ, Satoor SN, Ranjan AK, Pereirae Cotta MV, Joglekar MV. A protocol for measurement of noncoding RNA in human serum. *Exp Diabetes Res*. 2012;2012:168368. doi:10.1155/2012/168368
27. Corona-Meraz FI, Vazquez-Del Mercado M, Ortega FJ, Ruiz-Quezada SL, Guzman-Ornelas MO, Navarro-Hernandez RE. Ageing influences the relationship of circulating miR-33a and miR- 33b levels with insulin resistance and adiposity. *Diab Vasc Dis Res*. 2019;16(3):244–253. doi:10.1177/1479164118816659
28. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009;55(4):611–622. doi:10.1373/clinchem.2008.112797
29. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife*. 2015;4. doi:10.7554/eLife.05005
30. Schickel R, Boyerinas B, Park SM, Peter ME. MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. *Oncogene*. 2008;27(45):5959–5974. doi:10.1038/onc.2008.274
31. Yang J, Li Z, Wang L, et al. The role of non-coding RNAs (miRNA and lncRNA) in the clinical management of rheumatoid arthritis. *Pharmacol Res*. 2022;186:106549. doi:10.1016/j.phrs.2022.106549
32. Yue J, Lau TCK, Griffith JF, et al. Circulating miR-99b-5p as a novel predictor of erosion progression on high-resolution peripheral quantitative computed tomography in early rheumatoid arthritis: a prospective cohort study. *Int J Rheum Dis*. 2019;22(9):1724–1733. doi:10.1111/1756-185X.13644
33. Shen MY, Jiang BP, Zhang MF, et al. MicroRNA-143-3p ameliorates collagen-induced arthritis by polarizing naive CD4(+) T cells into Treg cells. *J Clin Lab Anal*. 2023;37(5):e24845. doi:10.1002/jcla.24845
34. Zhou LL, Zhu YM, Qian FY, Yuan CC, Yuan DP, Zhou XP. MicroRNA-143-3p contributes to the regulation of pain responses in collagen-induced arthritis. *Mol Med Rep*. 2018;18(3):3219–3228. doi:10.3892/mmr.2018.9322
35. Karimi L, Mansoori B, Shanebandi D, Mohammadi A, Aghapour M, Baradaran B. Function of microRNA-143 in different signal pathways in cancer: new insights into cancer therapy. *Biomed Pharmacother*. 2017;91:121–131. doi:10.1016/j.biopha.2017.04.060
36. Tavasolian F, Hosseini AZ, Soudi S, Naderi M. miRNA-146a improves immunomodulatory effects of msc-derived exosomes in rheumatoid arthritis. *Curr Gene Ther*. 2020;20(4):297–312. doi:10.2174/1566523220666200916120708
37. El-Bakry RM, Fadda SM, Mohamed RA, Abdelaleem EA. Circulating miR-146a −5p in Egyptian patients with rheumatoid arthritis. *Egypt J Immunol*. 2022;29(1):29–38.
38. Bae SC, Lee YH. MiR-146a levels in rheumatoid arthritis and their correlation with disease activity: a meta-analysis. *Int J Rheum Dis*. 2018;21(7):1335–1342. doi:10.1111/1756-185X.13338
39. Chen X, Xie L, Jiang Y, Zhang R, Wu WLCK. FOXC1 and hsa-miR-146a-5p as potential immune effector molecules associated with rheumatoid arthritis. *Biomarkers*. 2023;28(1):130–138. doi:10.1080/1354750X.2022.2150315
40. Tan H, Ma L, Qin T, et al. Myo6 mediates osteoclast function and is essential for joint damage in collagen-induced arthritis. *Biochim Biophys Acta Mol Basis Dis*. 2024;1870(1):166902. doi:10.1016/j.bbadis.2023.166902