Clinical and Experimental Medicine (2019) 19:47–53
https://doi.org/10.1007/s10238-018-0524-3

ORIGINAL ARTICLE

Upregulation of miR-221/222 expression in rheumatoid arthritis (RA) patients: correlation with disease activity

Amira S. Abo ElAtta[1] · Yasser B. M. Ali[1] · Iman H. Bassyouni[2] · Roba M. Talaat[1]

Received: 24 May 2018 / Accepted: 2 August 2018 / Published online: 21 August 2018

Abstract

miRNAs are noncoding RNA that play a critical role as fine regulators of gene expression at the posttranscriptional level within cells in numerous autoimmune diseases. miR-221/222 play a role in cancer by regulating cell proliferation, invasion and apoptosis. However, there have been insufficient studies on their role in rheumatoid arthritis (RA). This work is designed to analyze the miR-221/222 expression patterns in peripheral blood mononuclear cells (PBMCs) of patients with RA in comparison with healthy controls using quantitative RT-PCR, in a group of 30 RA patients and 20 healthy controls. The fold change of miR-221/222 expression in PBMCs was significantly elevated ($p < 0.01$) in RA patients compared with healthy controls. A positive correlation between expression levels of miR-221 and miR-222 was recorded ($r = 0.303$; $p < 0.05$). High miR-221/222 expression levels appeared to be elevated with high activity. miR-222 expression in high activity group of RA patients was significantly increased in relation to moderate ($p < 0.01$) and low ($p < 0.001$) activity ones with positive correlation ($r = 0.363$; $p < 0.05$) between the progress of disease activity and change in miR-222 expression level. ROC analysis showed a sensitivity of 70% and specificity of 75% for miR-221. In miR-222, the sensitivity of 80% and specificity of 70% were recorded. Our data shed some light on the role of miR-221/222 expression in RA patients, and their great potential value as new novel noninvasive biomarkers for disease detection. Therefore; further investigations are warranted to fully elucidate their role in rheumatoid.

Keywords Rheumatoid arthritis · MicroRNAs · miR-221 · miR-222

Introduction

Rheumatoid arthritis (RA) is a systemic, inflammatory, autoimmune disease, characterized by pursuing ruin of joint and deformation of bone that affects 0.5–2.0% regarding the human population global [1]. Women are 3 instances greater affected by RA than men. Recent publication in 2018 has studied epidemiology of RA in Egypt [2]. From 3219 RA patients, 84% were females indicating to a female predominance.

Age of disease onset ranged from 40 to 50 years, although it may affect people of any age. It is a disabling and onerous condition, which can lead to substantial loss of functioning and mobility if not adequately treated [3, 4]. The mechanisms worried into ailment initiation or progression are nevertheless incompletely understood, namely RA has a complicated factor triggered by means of several genes up to expectation engage together including environmental and stochastic factors [5].

MicroRNAs (miRNAs) are small, noncoding RNAs that are epigenetic regulators of cellular protein levels through destabilization of mRNA and/or inhibition of protein translation. They can target numerous mRNAs and thus affect simultaneously several biological pathways [6, 7]. miRNAs are highly abundant and show high stability in biological fluids, with changes in their levels correlating with disease

✉ Roba M. Talaat
roba.talaat@gebri.usc.edu.eg; robamtalaat@gmail.com

Amira S. Abo ElAtta
miro_200778@yahoo.com

Yasser B. M. Ali
yassermb@yahoo.com

Iman H. Bassyouni
imanbassyouni@yahoo.com

[1] Sadat City University, Sadat City, Menufyia Governerate, Egypt

[2] Department of Rheumatology and Rehabilitation, El-Kasr El-Aini Hospital, Faculty of Medicine, Cairo University, Cairo 12613, Egypt

prognosis and/or disease activity [8]. Recent studies have supported an important role for miRNAs in RA, and dysregulated miRNAs expression seems to contribute to the molecular mechanisms of the disease, and how they regulate pathways in RA and consider it as a new player for diagnostic and therapeutic purpose [9]. In a previous study, differential expression of circulating miR-223 and miR-16 was demonstrated in the sera of RA patients characterizing an early stage of the disease. They suggest miR-223 as a marker of disease activity and miR-16 and miR-223 as possible predictors for disease outcome [10]. Other studies proposed miR-146a [11], and miR-155 [12] as a biomarker of RA disease activity.

Despite the great deal of work that has been done to date, minority of the cases were the targets of miR-221/222 that control the two arms of RA: angiogenesis and inflammation. miR-221 and miR-222, are present alongside in a gene cluster placed on X chromosome (Xp11.3), comprise equal seed sequences separated by using 727 bases and are exceptionally conserved in vertebrates [13]. miR-221/222 have been found in healthy people to adjust integral physiological vascular procedures such as angiogenesis, vessel wound healing, vascular aging and atherosclerotic vascular remodeling [14–16]. Adaptive manner involving phenotypic and behavioral adjustments in vascular cells in response to vascular harm is contributed by using miR-221/222 [17], which regulates also the function of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) [18].

miR-221/222 are considered from the most critical oncogenes; they play crucial roles in pathogenesis of inflammatory diseases as atherosclerosis and RA by regulating the expression of different proteins [19, 20]. Although the potential contribution of miR-221/222 in RA inflammation and pathogenesis is not known yet, their role in RA remains to be fully elaborated. Thus, the aim of our study was to analyze the miR-221/222 expression patterns in peripheral blood mononuclear cells (PBMCs) of patients with RA in comparison with healthy controls using quantitative RT-PCR and their correlation with disease score and activity.

Patients and methods

Patient and study design

A group of 20 healthy donors with no history of autoimmune disease were included as control individuals, and 30 consecutive patients fulfilling an American college of rheumatology/European league against rheumatism collaborative initiative (ACR/EULAR) criteria for diagnosis of RA [21] were included. Demographic and clinical characteristics were collected through a structured interview and physical examination. They all attended the Rheumatology Department at El-Eini Hospitals, Cairo University. Patients with infection, malignancy as well as those with other autoimmune diseases were excluded from the study. All investigations were done in accordance with the Ministry of Health, Health and Human Ethical Clearance Committee guidelines for Clinical Researches. Cairo University local Ethics Committee approved the study protocol. All patients and healthy controls agreed to be enrolled in this study, and informed consent was obtained from all participants.

RA disease activity was assessed by using the Disease Activity Score 28 (DAS28) based on evaluation of 28 joints (tenderness and swelling joint count), rheumatoid factor (RF) and the erythrocyte sedimentation rate (ESR) [22]. Functional ability was assessed by using the modified version of the health assessment questionnaire (mHAQ) which was validated previously [23]. Radiographs of the hands and feet were reviewed for all patients. The evaluation of erosive disease was done by standard radiographs of both hands and feet at the assessment time. At least one definite erosive change on any of the hands or feet radiographs was sufficient for inclusion of the patient to the erosive group. Radiographic joint damage was further assessed according to sharp score with the number and size of bone erosions and the extent of joint space narrowing related to the cartilage damage being evaluated. Routine biochemistry tests were collected from patients' records.

PBMC collection and quantitative real-time RT-PCR

Heparinized venous blood was collected from each patient and control (5 ml) by venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation. Blood was diluted with phosphate-buffered saline (PBS; 1:1) and layered on Ficoll-Hypaque (1:3) and centrifuged at 1500 rpm for 30 min at 24 °C. The PBMCs were collected from the interface and washed two times with PBS and once with RPMI-1640 supplemented with L-glutamine (200 mM), penicillin (100 U/ml), streptomycin (100 µg/ml) and HEPES buffer (1 M) (all from Biowest SAS, Nuaillé, France) [24].

Total RNA was isolated from freshly obtained PBMCs using the TRIzol isolation protocol (Life Technologies Ltd. UK). The purity and concentration of RNA were assessed by NanoDrop™ 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the integrity was checked by agarose gel electrophoresis. A total of 100 ng of each RNA sample was used for quantitative real-time RT-PCR (qRT-PCR). cDNA was reverse-transcribed using miScriptII RT kit (Qiagen, Valencia, CA, USA) according to the manufacturer's directions (catalog numbers 218061). qRT-PCR for the detection of Hs_miR-221 and Hs_miR-222 was carried out in 25-µl PCR reactions using the miScript SYBR Green PCR Kit (catalog numbers 218073) and miScript

Primer Assays (Qiagen) according to the manufacturer's protocol. The thermal cycling conditions comprised 95 °C for 15 min as initial activation step, then 95 °C for 15 s, followed by 55 °C for 30 s and 70 °C for 30 s in 40 cycles. The expression of the U6B small nuclear RNA (RNU6B) was used as endogenous control for data normalization. Differences in the Ct values (Ct) between the tested miRNA and RNU6B cDNA were calculated to determine the relative expression levels, using the following formula: $\Delta\Delta Ct = \Delta Ct$ of the tested sample $-\Delta Ct$ of the control sample. The value of each control sample was set at 1 and was used to calculate the fold change in target genes by using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were performed using SPSS version 19 (SPSS, Inc., Chicago, IL). Data were statistically described in terms of mean ± standard deviation (± SD), frequencies when appropriate. Spearman's test was used for correlation analysis. Student t test was used for comparison of numerical variables between the study groups for quantitative variables, One-way ANOVA test was used. Sensitivity versus the false positive frequency (one-specificity) for the scoring system was analyzed by a receiver-operated characteristic (ROC) curve. Probability values less than 0.05 were considered significant.

Table 1 Demographic and laboratory characteristics of patients with rheumatoid arthritis (RA)

Parameter	Mean ± SD
Age (years)	39.10 ± 10.43
Female/male	27/3
Disease duration (years)	3.49 ± 2.46
Tender joints (of 28)	8.53 ± 6.75
Swollen joints (of 28)	4.03 ± 4.45
DAS-28	4.36 ± 1.00
mHAQ	0.64 ± 0.52
Sharp score of erosions (0–170)	37.26 ± 33.95
Parameter	No (%)
RF +ve	18 (60)
Subcutaneous nodules	5 (16.6)
Extra-articular disease	8 (26.6)
Laboratory parameters	Mean ± SD
ESR (mm/h)	39.86 ± 21.97
Hemoglobin (g/dl)	12.00 ± 1.47
TLC (× 1000/µl)	8.02 ± 3.412
Platelets (× 1000/µl)	269.73 ± 74.13
Creatinine (mg/dl)	0.72 ± 0.30
ALT (U/L)	21.20 ± 17.42
AST (U/L)	22.03 ± 9.99

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *DAS28* disease activity score 28, *ESR* erythrocytes sedimentation rate, *HB* hemoglobin, *RF* rheumatoid factor, *TLC* total leukocyte count

Results

Patient's characteristics

Clinical, demographic and laboratory data are presented in Table 1. Patients and healthy controls were age- and sex-matched. Of the 30 RA patients who were enrolled at this study, 27 were females and 3 were males with mean age of 39.10 ± 10.43 years. Age of disease onset ranged from 18 to 60 years, and disease duration ranged from 1 to 9 years. Patients were classified as having active or inactive disease on the basis of the DAS28 into: 4 patients had low active disease (DAS28 < 3.2), 20 had moderately active disease (DAS28 from 3.2 to < 5.1), and 6 patients had high disease activity (DAS28 > 5.1). Twenty-three patients were under treatment of disease-modifying antirheumatic drugs (DMARDs); 18 patients received methotrexate (MTX), and 5 patients received Leflunomide (LFN). The rest of RA patients [7] were untreated.

miR-221-222 PBMCs expression levels

Using RT-qPCR analysis, miR-221/222 expression levels in PBMCs of patients with RA and healthy controls were determined. It was found that the fold change of miR-221/222 expression in PBMCs was significantly elevated ($p < 0.01$) in RA patients compared with those in healthy controls (Fig. 1a). A positive correlation between miR-221 expression level and miR-222 expression level was recorded ($r = 0.303$; $p < 0.05$) (Fig. 1b). Although a slight increase in miR-221 in MTX-treated patients was found, no significant change in both miR-221/222 expression levels was observed between treated and untreated groups (Table 2).

To examine the relationship between RA disease activity and miRNA expression levels, patients were classified into low, moderate and high disease activity based on DAS28 values. High miR-221/222 expression levels appeared to be elevated with high activity, whereas low expression level coincided with low disease activity. As shown in Fig. 2a, a significant increase was detected in miR-222 expression in high activity group of RA patients in relation to moderate ($p < 0.01$) and low ($p < 0.001$) activity ones. Although a miR-221 expression level was increased in RA patients with more active stage, the elevation was not statistically significant. A positive correlation ($r = 0.363$; $p < 0.05$) was

recorded between the progress of disease activity and change in miR-222 expression level (Fig. 2b).

After ROC analysis (Fig. 3a, b), the area under the curve (AUC) for miR-221 was 0.724 (95% confidence interval [CI], CI 0.584–0.864), and AUC of 0.768 (95% CI 0.629–0.908) for miR-222. Among different cutoff values from the ROC analysis, a cutoff value of 2.265 was selected, as the sensitivity of 70% and specificity of 75% at the chosen cutoff were optimal for miR-221. In miR-222, a cutoff value of 0.595 was selected, as the sensitivity of 80% and specificity of 70%.

Discussion

miRNAs are involved in the pathophysiological mechanisms-related human diseases [25]. miRNAs are of interest due to their critical role as fine regulators of gene expression at the posttranscriptional level within cells in numerous diseases, which makes them potential targets in the treatment of different diseases. Their role in many human autoimmune diseases, such as RA, has been evaluated since the last decade [26].

The studies that talk about potential role of miRNAs as molecular biomarkers for diagnosis and prognosis in RA have been expanded. A lot of affective expressed miRNAs in circulation or inflamed joints in RA have been identified. That suggests their potential to be used as biomarkers that help to establish or approve a diagnosis, adjust the degree of immunologic activity or inflammation, or provide prognostic information regarding disease progression and severity [27]. The accumulating evidence has demonstrated that the expression of miRNAs in RA, including miR-146a, miR-155, miR-16, miR-23b, miR-203, miR-124a, miR-346, miR-223 as well as miR-34a, was altered in synovial

Table 2 miR-221/222 expression in treated and untreated RA patients

Treatment (*N*)	miR-221 (mean ± SE)	miR-222 (mean ± SE)
Untreated (7)	6.05 ± 3.04	12.94 ± 9.20
MTX (18)	42.08 ± 15.73	15.05 ± 4.90
LFN (5)	21.07 ± 9.40	34.51 ± 17.62

MTX methotrexate, *LFN* leflunomide

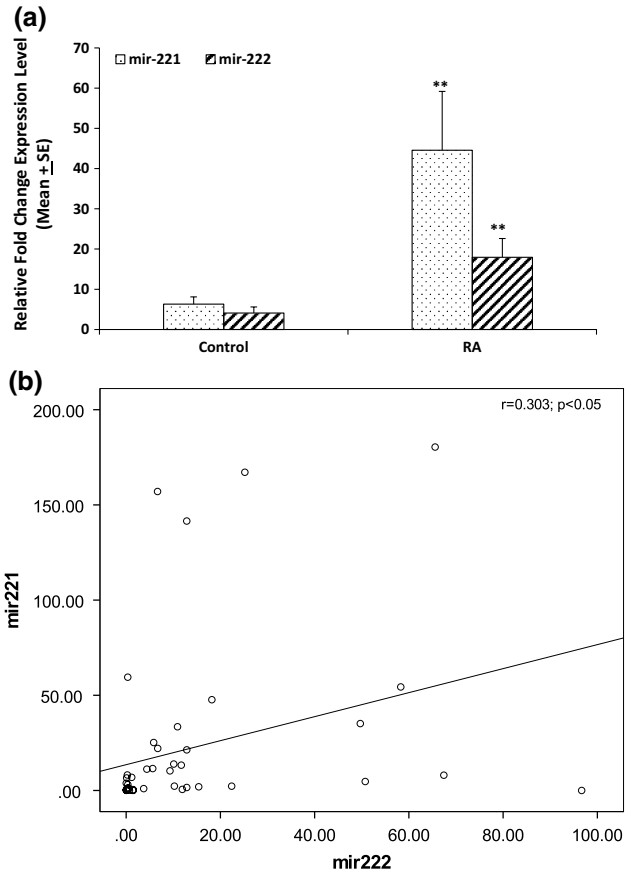

Fig. 1 Relative fold change expression levels of miR-221/222 in rheumatoid arthritis (RA) and normal controls. (*) denotes significant from healthy controls. (**) $p < 0.001$

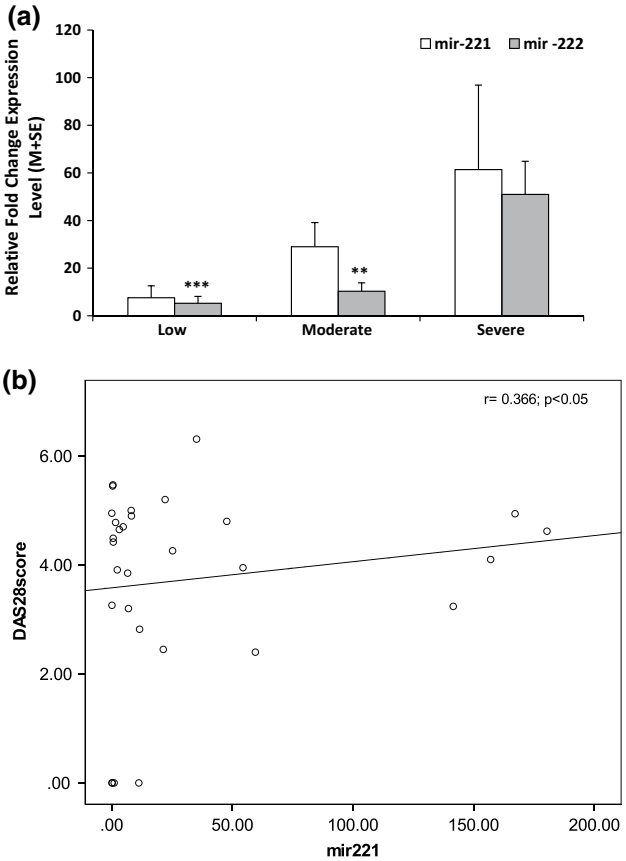

Fig. 2 Correlation between miR-221/222 in RA patients

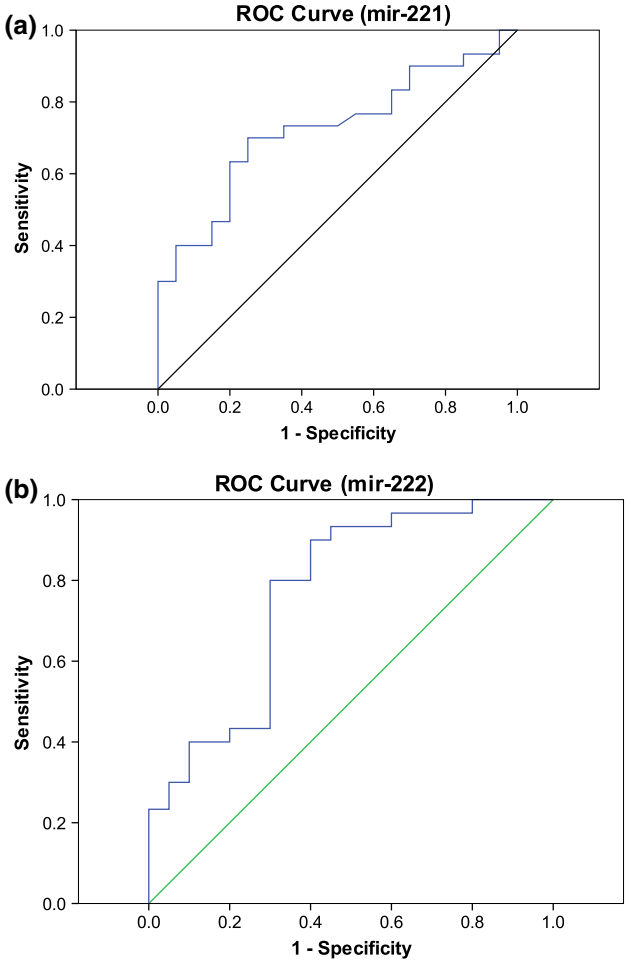

Fig. 3 Area under curve of receiver operating characteristic of the miR-221 (**a**) and miR-222 (**b**) to discriminate RA patients from normal controls

fibroblasts, peripheral blood mononuclear cells, and T cells from RA patients [28–34]. It has been reported that this abnormal miRNA expression is associated with abnormal innate immunity, inflammation, cell proliferation and cell apoptosis [35–38]. Thus, the suppression of over-expressed miRNAs or reconstitution of the expression by restoration of silenced miRNAs is a therapeutic strategy in the treatment of RA [39].

Pauley et al. [40] have established a significant study that demonstrates that miRNA expression in RA PBMCs may mimic conditions in synovial tissue and thus makes us able to avoid the need of synovial tissue samples, allowing the analysis of larger patient populations. Accordingly, the present study showed that PBMCs miRNAs (miR-221/222) expression was elevated in RA patients compared with those in healthy individuals. The ROC curves of miR-221/222 reflected a good separation between control and RA patients, with 70% sensitivity/75% specificity for miR-221 and 80% sensitivity/70% specificity for miR-222. To examine the relationship between RA disease activity and miRNA expression levels, patients were classified into active/or inactive according to DAS28. miR-221/222 were increased with disease activity.

Our finding is similar to Pandis et al. [41]; they confirmed that miR-221/222 have been found to be over-expressed in RA synovial fibroblasts, serum and synovial tissues of RA patients. Yang and Yang [20] suggested that miR-221 is relevant to RA pathogenesis and that it possibly has a crucial role in RA initiation, development and progression. Their findings of this study provided evidence that miR-221 expression was increased in patients with RA and that inhibition of miR-221 suppressed pro-inflammatory cytokines. Furthermore, down regulation of miR-221 in fibroblast-like synoviocytes could significantly inhibit the expression of pro-inflammatory cytokines and chemokine that lead to inhibition of cell overrun and migration, and inhibit vascular endothelial growth factor (VEGF), matrix metalloproteinase-3 (MMP-3) and matrix metalloproteinase-9 (MMP-9) that induce cell apoptosis [42, 43]. miR-221 over-expression in inhibited adiponectin-stimulated nitric oxide (NO) in HUVECs indicated that miR-221 targeted AdipoR1 for regulation of endothelial inflammatory response [44].

Our patient cohort is mainly composed of females (27 females vs 3 males). This is consistent with previous studies which documented that the majority of autoimmune diseases as RA is predominate in females [2]. From factors that can play an important role in sex bias in RA, the contribution of epigenetic medications and skewed X chromosome inactivation to RA female predominance has been previously studied [45–47]. The human X chromosome is highly enriched in miRNAs as compared to the Y chromosome, with 116 X-linked miRNAs against only two Y-linked miRNAs. Six miRNAs including miR-221, miR-222, miR-98, miR-532, miR-106a and miR-92a have sexual dimorphisms. Kukurba et al. [48] demonstrated that genes on the X chromosome are more likely to have sex-specific expression compared to genes on the autosomes. Regarding miR-221/222, Khalifa et al. [49] showed that miR-221, miR-222, miR-532, miR-106a and miR-98 expression levels were significantly different between RA and controls only when allotment to the sex. Moreover, the expression level of miR-222, miR-532, miR-98 and miR-92a was significantly different between RA female and male.

In general, very limited studies on circulated miR-221/222 are found. In RA, studies on circulated miR-221/222 are more restricted and the majority of them more focused on SF. Previous data in different diseases discussed the circulating expression levels of miR-221/222 and showed upregulation of them. Teixeira et al. [50] identified the upregulation of plasma miR-221/222 as potential noninvasive biomarkers for the detection of renal cell carcinoma (RCC) RCC. El-Garem et al. [51] that dealt with circulating

miR-221 signature in Egyptian patients with chronic hepatitis C related hepatocellular carcinoma. They explore serum miR-221 to serve as one of the novel noninvasive biomarkers of HCC.

Taken together, the findings of the present study provided evidence that miR-221/222 expression were increased in patients with RA; they have great potential value as new novel noninvasive biomarkers for RA detection. Both miR-221 and miR-222 were increased with RA disease activity. Each identified miRNA in RA disease opens the door slightly not only in terms of diagnostic and/or prognostic marker, but also for therapy in the future.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Informed consent All patients and healthy controls agreed to be enrolled in this study, and informed consent was obtained from all participants.

References

1. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet. 2001;358:903–11.
2. Sakr BR, Elfishawi MM, ElArousy MH, et al. Rheumatoid arthritis: a single center Egyptian experience. Immunol Invest. 2018;47(3):293–302.
3. Baillet A, Trocmé C, Berthier S. Synovial fluid proteomic fingerprint: S100A8, S100A9 and S100A12 proteins discriminate rheumatoid arthritis from other inflammatory joint diseases. Rheumatology. 2010;49(4):671–82.
4. Zendman AJ, van Venrooij WJ, Pruijn GJ. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology. 2006;45:20–5.
5. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet. 2009;373:659–72.
6. Chekulaeva M, Filipowicz W. Mechanisms of miRNA-mediated posttranscriptional regulation in animal cells. Curr Opin Cell Biol. 2009;21:452–60.
7. Xu WD, Lu MM, Pan HF, et al. Association of microRNA-146a with autoimmune diseases. Inflammation. 2012;35:1525–9.
8. Wang Y, Li Z, He C, et al. MicroRNAs expression signatures are associated with lineage and survival in acute leukemias. Blood Cells Mol Dis. 2010;44:191–7.
9. Li J, Wan Y, Guo Q, et al. Altered microRNA expression profile with miR-146a upregulation in CD4+ T cells from patients with rheumatoid arthritis. Arthritis Res Ther. 2010;12:R81.
10. Filková M, Aradi B, Senolt L, Ospelt C, Vettori S, Mann H, Gay S, et al. Association of circulating miR-223 and miR-16 with disease activity in patients with early rheumatoid arthritis. Ann Rheum Dis. 2014;73(10):1898–904.
11. Chen ZZ, Zhang XD, Chen Y, Wu YB. The role of circulating miR-146a in patients with rheumatoid arthritis treated by *Tripterygium wilfordii* Hook F. Medicine (Baltimore). 2017;96(20):e6775.
12. Long L, Yu P, Liu Y, Wang S, Li R, et al. Upregulated microRNA-155 expression in peripheral blood mononuclear cells and fibroblast-like synoviocytes in rheumatoid arthritis. Clin Dev Immunol. 2013;2013:296139.
13. Miao CG, Yang YY, He X, et al. New advances of microRNAs in the pathogenesis of rheumatoid arthritis, with a focus on the crosstalk between DNA methylation and the microRNA machinery. Cell Signal. 2013;25:1118–25.
14. Vicente R, Noel D, Pers YM, Apparailly F, Jorgensen C. Deregulation and therapeutic potential of microRNAs in arthritic diseases. Nat Rev Rheumatol. 2016;12:211–20.
15. Song Z, Li G. Role of specific microRNAs in regulation of vascular smooth muscle cell differentiation and the response to injury. J Cardiovasc Transl Res. 2010;3:246–50.
16. Kuehbacher A, Urbich C, Dimmeler S. Targeting microRNA expression to regulate angiogenesis. Trends Pharmacol Sci. 2008;29:12–5.
17. Chistiakov DA, Sobenin IA, Orekhov AN, Bobryshev YV. Human miR-221/222 in physiological and atherosclerotic vascular remodeling. Biomed Res Int. 2015;2015:354517.
18. Rippe C, Blimline M, Magerko KA, et al. microRNA changes in human arterial endothelial cells with senescence: relation to apoptosis, eNOS and inflammation. Exp Gerontol. 2012;47:45–51.
19. Stenmark KR, Yeager ME, El Kasmi KC, et al. The adventitia: essential regulator of vascular wall structure and function. Annu Rev Physiol. 2013;75:23–47.
20. Yang S, Yang Y. Downregulation of microRNA221 decreases migration and invasion in fibroblast-like synoviocytes in rheumatoid arthritis. Mol Med Rep. 2015;12:2395–401.
21. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569–81.
22. Prevoo ML, van 't Hof MA MA, Kuper HH, et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995;38(1):44–8.
23. Pincus T, Yazici Y. Bergman M. Development of a multidimensional health assessment questionnaire (MD-HAQ) for the infrastructure of standard clinical care. Clin Exp Rheumatol. 2005;23(Suppl 39):S19–28.
24. Talaat RM, El-Bassiouny AI, Osman AM, Yossif M, Charmy R, Al-Sherbiny MM. Cytokine secretion profile associated with periportal fibrosis in *S. mansoni*-infected Egyptian patients. Parasitol Res. 2007;101:289–99.
25. Lian JB, Stein GS, van Wijnen AJ, et al. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol. 2012;8(4):212–27.
26. Duroux-Richard I, Jorgensen C, Apparailly F. miRNAs and rheumatoid arthritis—promising novel biomarkers. Swiss Med Wkly. 2011;141:w13175.
27. Zhu S, Pan W, Song X, et al. The microRNA miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TAB 2, TAB 3 and IKK-α. Nat Med. 2012;18(7):1077–86.
28. O'Connell RM, Kahn D, Gibson WS, et al. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity. 2010;33:607–19.
29. Baxter D, McInnes IB, Kurowska-Stolarska M. Novel regulatory mechanisms in inflammatory arthritis: a role for microRNA. Immunol Cell Biol. 2012;90:288–92.
30. Jimenez SA, Piera-Velazquez S. Potential role of human-specific genes, human specific microRNAs and human-specific non-coding regulatory RNAs in the pathogenesis of systemic sclerosis and Sjogren's syndrome. Autoimmun Rev. 2013;12:1046–51.

31. Bottini N, Firestein GS. Epigenetics in rheumatoid arthritis: a primer for rheumatologists. Curr Rheumatol Rep. 2013;15(11):372.
32. Viatte S, Plant D, Raychaudhuri S. Genetics and epigenetics of rheumatoid arthritis. Nat Rev Rheumatol. 2013;9(3):141–53.
33. Glant TT, Mikecz K, Rauch TA. Epigenetics in the pathogenesis of rheumatoid arthritis. BMC Med. 2014;12:35.
34. Reveille JD. Genetic studies in the rheumatic diseases: present status and implications for the future. J Rheumatol Suppl. 2005;72:10–3.
35. Zhou Q, Haupt S, Kreuzer JT, et al. Decreased expression of miR-146a and miR-155 contributes to an abnormal Treg phenotype in patients with rheumatoid arthritis. Ann Rheum Dis. 2015;74(6):1265–74.
36. Churov AV, Oleinik EK, Knip M. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. Autoimmun Rev. 2015;14(11):1029–37.
37. Kim T-H, Choi SJ, Lee YH, Song GG, Ji JD. Gene expression profile predicting the response to anti-TNF treatment in patients with rheumatoid arthritis; analysis of GEO datasets. Joint Bone Spine. 2014;81(4):325–30.
38. Park HJ, Kim DH, Lim SH, et al. Insights into the role of follicular helper T cells in autoimmunity. Immune Netw. 2014;14(1):21–9.
39. Ruedel A, Dietrich P, Schubert T, Hofmeister S, Hellerbrand C, Bosserhoff AK. Expression and function of microRNA-188-5p in activated rheumatoid arthritis synovial fibroblasts. Int J Clin Exp Pathol. 2015;8(6):6607–16.
40. 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.
41. Pandis I, Ospelt C, Karagianni N, Denis MC, Reczko M, Camps C, et al. Identification of microRNA-221/222 and microRNA-323-3p association with rheumatoid arthritis via predictions using the human tumour necrosis factor transgenic mouse model. Ann Rheum Dis. 2012;71:1716–23. https://doi.org/10.1136/annrheumdis-2011-200803.
42. Zhang X, Shao S, Geng H, et al. Expression profiles of six circulating microRNAs critical to atherosclerosis in patients with subclinical hypothyroidism: a clinical study. J Clin Endocrinol Metab. 2014;99:E766. https://doi.org/10.1210/jc.2013-1629
43. Minami Y, Satoh M, Maesawa C, et al. Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. Eur J Clin Invest. 2009;39:359–67. https://doi.org/10.1111/j.1365-2362.2009.02110.x.
44. Chen CF, Huang J, Li H, Zhang C, Huang X, Tong G, et al. microRNA-221 regulates endothelial nitric oxide production and inflammatory response by targeting adiponectin receptor 1. Gene. 2015;565:246–51.
45. Migeon BR. Females are mosaics: X inactivation and sex differences in disease. New York: Oxford University Press, Inc.; 2007.
46. Ozcelik T. X chromosome inactivation and female predisposition to autoimmunity. Clin Rev Allergy Immunol. 2008;34:348–51.
47. Carmona L, Cross M, Williams B, Lassere M, March L. Rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2010;24:733–45.
48. Kukurba KR, Parsana P, Balliu B, Smith K, Zappala Z, Knowles DA, et al. Impact of the X Chromosome and sex on regulatory variation. Genome Res. 2016;26:768–77.
49. Khalifa O, Pers YM, Ferreira R, Sénéchal A, et al. X-linked miRNAs associated with gender differences in rheumatoid arthritis. Int J Mol Sci. 2016;17(11):1852.
50. Teixeira AL, Ferreira M, Silva J, Gomes M, et al. Higher circulating expression Levels of miR-221 associated with poor overall survival in renal cell carcinoma patients. Tumour Biol. 2014;35(5):4057–66.
51. El-Garem H, Ammer A, Shehab H, Shaker O, et al. Circulating microRNA, miR-122 and miR-221 signature in Egyptian patients with chronic hepatitis C related hepatocellular carcinoma. World J Hepatol. 2014;6(11):818–24.