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Serum miR-210 and miR-155 expression levels as novel biomarkers for rheumatoid arthritis diagnosis

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ABSTRACT

Background: MicroRNAs play a crucial role in the regulation of immune response. We hypothesised roles for serum miR-210 and miR-155 in the diagnosis of rheumatoid arthritis (RA) and relationships with the clinical and laboratory variables including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP) antibodies, tumour necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β).

Methods: MiR-210 and miR-155 levels were identified by real-time polymerase chain reaction (PCR). TNF-α and IL-1β were measured by enzyme-linked immunosorbent assay and routine markers by standard techniques in 100 patients with RA and 100 individuals as healthy controls. Disease activity in the patients was assessed by DA-S28.

Results: MiR-210 was lower in RA compared to controls [median/IQR 0.96 (0.8–1.24) vs. 4 (1.28–3.93), $p < 0.001$]. miR-210 correlated inversely with clinical and laboratory variables including TNF-α and IL-1β (both $r = -0.96$, $p < 0.001$). MiR-155 expression was increased in RA compared to controls [median/IQR 6 (3.5–8.1) vs. 1.0 (0.95–1.6), $p < 0.001$] and correlated with TNF-α and IL-1β (both $r = 0.94$, $p < 0.001$). In multivariate analysis, miR-210 and miR-155 were both independent diagnostic markers for RA, and both were associated with RA disease activity.

Conclusion: Serum miR-210 and miR-155 levels are independent diagnostic markers for RA, outperforming several routine indices and reflect disease activity. Thus, miR-210 and miR-155 might serve as non-invasive biomarkers for the diagnosis of RA.

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Introduction

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory, autoimmune disease that affects primarily the articular cartilage and bone. The most important features of RA are persistent inflammation, cartilage erosion with subsequent joint destruction and deformity [1]. Untreated patients have a progressive course resulting in short- and long-term disability, so early treatment can prevent severe disability and leads to patient benefits [2,3]. Therefore, biomarkers for the diagnosis and prediction of therapeutic outcomes are needed, enabling clinicians to treat patients as early as possible with the best therapeutic approach. Pro-inflammatory cytokines play an important role in the pathophysiology of RA: the release of TNF-α and IL-1β causes synovial inflammation, and cytokines promote the production of acute-phase proteins (such as CRP) and development of cardiovascular disease and anaemia [4].

MicroRNAs are small (18–22 nucleotides) non-coding RNA molecules that have an important role in the regulation of gene expression. It has been reported that around 30% of human genes are regulated by miRNAs [5]. They mediate this regulation either by mRNA degradation or by translational repression and possess diverse functions in regulating some cellular processes, including cellular differentiation, proliferation, apoptosis as well as cancer development [6–8]. Moreover, miRNAs have important roles in regulating the immune responses and the development of autoimmunity [9,10].

Increasing evidence has linked circulating miRNAs with the diagnosis and progression of a variety of diseases, including cardiovascular disease, infectious diseases and some cancers [11–13]. Biochemical and genetic studies revealed the physiological functions of individual miRNAs in immunity [14]. Altered expression of miRNAs in synovial tissue, synovial fibroblasts, peripheral blood mononuclear cells, osteoclasts and isolated

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T lymphocytes results in the degradation of extracellular matrix, inflammation and invasive behaviour of resident cells [15–17]. Furthermore, several studies have supported an aberrant miRNA expression in different cell types in RA, thus regulating specific pathways involved in pathogenesis [18–20]. It has been reported that miR-210 inhibit the activation of $NF_{-\kappa}$ B pathway, an important regulator of the immune response, inflammation as well as cell survival [21,22]. In clinical and experimental arthritis models, Kurowska-Stolarska et al. [23] showed that miR-155 plays an important role in the proinflammatory activation of myeloid cells and the development of antigen-derived inflammatory arthritis.

We hypothesise that miR-210 and miR-155 are more effective in the diagnosis of RA than other laboratory markers and are linked to disease activity. Moreover, we hypothesise that these miRNAs could play an important role in the pathogenesis of RA through a link with TNF-α and IL-1β.

Materials and methods

The study was carried out on 200 individuals: 100 patients with RA (disease duration 8.0 ± 3.0 years) and 100 healthy age matched as controls. Patients were diagnosed according to 1987 revised American association rheumatism criteria for the classification of rheumatoid arthritis [24]. They were recruited from the outpatient clinics and inpatient units of Rheumatology and Rehabilitation Department, Zagazig University Hospitals, Faculty of Medicine, Zagazig, Egypt. We excluded patients with hepatic, diabetes, renal or malignant diseases. Patients were subjected to full history taking and thorough clinical examination. Patients medical records were reviewed for the documentation of clinical and laboratory data. All patients received non-steroidal anti-inflammatory drugs (NSAIDs) daily and 68% took steroids. DMARDs alone or in combination with other drugs were administered in 80% of subjects and 15% of patients were treated with biologics (infliximab and humira). RA disease activity was assessed using the Disease Activity Score (DAS-28 score). DAS-28 depends on the following parameters (swollen joint count (SJC), tender joint count (TJC), erythrocyte sedimentation rate (ESR)). For DAS-28 score, low disease activity was defined as DAS-28 ≤ 3.2, moderate as 3.2 < DAS-28 ≤ 5.1, high as DAS-28 > 5.1 and remission as DAS-28 < 2.6 [25]. The median/IQR of SJC, TJC and DAS-28 were 12 (8–15), 16 (11–20) and 4 (3–6), respectively. The control group had no family history of RA, did not show any clinical or laboratory signs of autoimmune diseases and they were free of any treatment. This study was approved by the Ethics Committee of Zagazig University and all subjects were included in the study after giving their informed consent after explanation of the purpose and procedures of the study.

After an overnight fast, venous blood samples were collected by venipuncture under complete aseptic conditions and divided into two portions: 5 ml of blood was collected into specific tubes for obtaining serum. Serum was stored at –80 °C till analysis. Another 1.6 ml was collected in tubes contain 0.4 ml trisodium citrate (109 mmol/l) and used to measure ESR according to Westergren [26]. Levels of serum CRP and rheumatiod factor (RF) were measured by immunturbidimetric assay on a Roche/Hitachi cobas c system (c501) autoanalyzer (Roche Diagnostics, Mannheim, Germany) using CRPL3 and RFII reagents respectively. Anti-CCP antibodies were measured on a Cobas e601 immuno-autoanalyzer (Roche Diagnostics) that adopts an electrochemiluminescence immunoassay (ECLIA) technique using dedicated reagents. TNF-α and IL-1β were measured by ELISA using Quantikine Kits (R&D Systems, Minneapolis, MI, USA).

MiRNAs were extracted from 400 µl of serum using the miRNeasy kit (Qiagen, Valencia, CA, USA), quantified spectrophotometrically at 260 nm and stored at –80°C. RNA was reverse transcribed to cDNA with miScript II RT Kit (Qiagen, CA). For real-time PCR of miR-210 and miR-155, the amplification was performed using StepOnePlus™ RealTime PCR system (Applied Biosystems Inc., Foster, CA). Syn-cel-miR-39 miScript miRNA Mimic was used as an internal control for data normalisation. Real-time PCR was performed using miScript Primer Assays [containing miRNA-specific forward primers: miRNA-210 (Cat. No. MS00003801); miRNA-155 (Cat. No MS00031486); Syn-cel-miR-39 (Cat. No MSY0000010)] and miScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA) according to manufacturer's protocol with the manufacture-provided miScript Universal primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix. PCR was performed in a mixture containing 10 µl 2× QuantiTect SYBR Green PCR Master Mix (Qiagen, USA), 0.5 µl of each primer (100 pmol/ µl), 5 µl cDNA and water to adjust the final volume to 20 µl. PCR was performed under the following cycling conditions: denaturation at 95 °C for 15 min, followed by 40 cycles at 94 °C for 15s; 55 °C for 30s and at 70 °C for 30 s. Each measurement was performed in duplicate. Melting curves were performed to confirm the specificity of PCR products. MiRNAs expression levels were determined by using ΔΔCT method where the Ct of the target miRNAs were normalised to the Ct of Syn-cel-miR-39 (internal control). Relative changes of gene expression were calculated from the equation $2^{-\Delta\Delta Ct}$.

The intra-assay CV was 0.7% and 0.8% for miR-210 and miR-155, respectively. The interassay CV of miR-210 and miR-155 was 2.3% and 2.5%, respectively. These values indicate that the techniques used in this study (RNA isolation and qRT-PCR) are reproducible. Data were analysed using SPSS statistical package version 17 (SPSS Inc., Chicago, IL, USA). Student's *t*-test and the chi square (x^2) test were used for comparison between groups. Mann–Whitney U-tests were used for non-normally distributed data. Correlations were assessed by Spearman's rank correlation. Contrast hypothesis test [27] was used to test the trend in continuous quantitative data. A *p*-value < 0.05 was considered significant.

Results

Clinical, demographic and laboratory data are presented in Table 1. Patients and controls were age and sex matched. Serum miR-210 levels were significantly lower in patients with RA compared to healthy control subjects, whilst miR-155 was higher in RA compared to controls. Unsurprisingly, all other laboratory indices were higher in RA than in controls. Multivariate regression analysis revealed that both miR-210 and miR-155, but not any other laboratory index, were independent diagnostic markers for RA (Table 2). Correlation of miRNAs with clinical and laboratory data in RA patients are shown in Table 3. Serum miR-210 correlated negatively, and miR-155 positively with ESR, CRP, SJC, TJC, DAS-28, RF, anti-CCP, TNF-α and IL-1β levels. No correlation was found between miRNAs expressions and other studied parameters including age and disease duration.

When stratifying RA patients according to disease activity (DAS-28 score) (Table 4), levels of miR-210 fell, whilst levels of miR-155, RF and IL-1β increased with increased disease activity. There was no significant difference in anti-CCP and TNF-α levels between different subgroups. When stratifying RA patients according to the type of treatment, there was no significant difference in miR-210 levels between patients treated with DMARDs or biologics (0.9 ± 0.3 vs. 1.0 ± 0.2, $p = 0.63$). Furthermore, miR-155 levels showed no significant difference between patients treated with DMARDs or biologics (6.7 ± 2.4 and 6.3 ± 2.5; $p = 0.51$).

Table 1. Demographic and laboratory data.

Variable	RA $N = 100$	Control $N = 100$	P value
Age (years)	51.0 ± 7.0	51.0 ± 6.0	0.51
Sex male/female	26 (26)/74 (74)	34 (34)/66 (66)	0.22
ESR (mm/h)	52 (36–64)	13 (10–13)	<0.001
CRP (mg/l)	34 (22–41)	10 (7–11)	<0.001
RF (IU/ml)	150 (99–179)	23 (14–56)	<0.001
Anti-CCP (U/ml)	120 (102–242)	10 (8–42)	<0.001
TNF-α (pg/ml)	35 (21–43)	12 (9–15)	<0.001
IL-1β (pg/ml)	38 (24–46)	18 (15–23)	<0.001
MiR-210	0.96 (0.8–1.2)	4 (1.2–3.9)	<0.001
MiR-155	6 (3.5–8.1)	1.0 (0.9–1.6)	<0.001

Notes: Data presented as mean ±SD, median (IQR) or n (%). ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; RF, rheumatoid factor; Anti CCP, anti-cyclic citrullinated peptide antibodies; TNF, tumour necrosis factor; IL-1β, interleukin 1β.

Table 2. Multivariate regression analysis for independent factors for detecting RA.

Parameters	Odds ratio	95%CI	P value
ESR	1.19	0.53–3.42	0.43
CRP	0.99	0.63–5.66	0.21
RF	0.97	0.32–12.33	0.45
Anti-CCP	1.12	0.23–7.88	0.17
TNF-α	1.06	0.73–5.66	0.11
IL-1β	1.10	0.02–11.45	0.09
miR-210	0.31	0.001–0.56	0.001
miR-155	2.30	1.5–7.8	0.009

Note: CI = confidence interval, see Table1 for other abbreviations.

Table 3. Spearman rank correlations (r) between serum miRNAs and clinical and laboratory data in RA patients.

Variable	miR-210	miR-155
TNF-α	$r = -0.96$: $p < 0.001$	$r = 0.94$: $p < 0.001$
IL-1β	$r = -0.96$: $p < 0.001$	$r = 0.94$: $p < 0.001$
Anti-CCP	$r = -0.95$: $p < 0.001$	$r = 0.94$: $p < 0.001$
SJC	$r = -0.93$: $p < 0.001$	$r = 0.90$: $p < 0.001$
TJC	$r = -0.94$: $p < 0.001$	$r = 0.91$: $p < 0.001$
DAS28	$r = -0.75$: $p < 0.001$	$r = 0.70$: $p < 0.001$
RF	$r = -0.54$: $p < 0.001$	$r = 0.48$: $p < 0.001$
CRP	$r = -0.35$: $p < 0.001$	$r = 0.32$: $p = 0.001$
ESR	$r = -0.28$: $p = 0.005$	$r = 0.27$: $p = 0.006$
Age	$r = 0.10$: $p = 0.33$	$r = -0.01$: $p = 0.90$
Disease duration	$r = 0.07$: $p = 0.50$	$r = -0.08$: $p = 0.86$

Notes: CI = confidence interval, SJC = swollen joint count, TJC = tender joint count. See Table 1 for other abbreviations.

Discussion

Rheumatoid arthritis (RA) is one of the leading causes of chronic morbidity in the developed world. Often seen as a minor health problem despite potentially fatal systemic manifestations, if untreated, it can lead to extensive damage of the cartilage and bone, causing deformity and disability [1,2,28]. Therefore, new biomarkers for RA diagnosis are urgently needed.

Recently, several miRNAs have been identified as playing important roles in the regulation of immune responses and the development of autoimmune disorders including RA [29,30]. In contrast to cellular or tissue miRNAs, the expression profile of circulatory miRNAs in RA has not been fully investigated. Therefore, we investigated levels of serum miR-210 and miR-155 in 100 patients with RA and 100 healthy controls. We also investigated the association of miR-210 and miR-155 with proinflammatory cytokines (TNF-α and IL-1β) as the precise role of miRNA regulation of proinflammatory cytokines in RA needs to be explored. Our principle observation is lower serum levels of miR-210 and higher levels of serum miR-155 in

Table 4. Change in levels of studied parameters in the sera of patients with different disease activities.

	DAS-28 score				
	Remission $N = 14$	Low disease activity $N = 20$	Moderate disease activity $N = 38$	High disease activity $N = 28$	P value
RF (IU/ml)	123 (56–149)	145 (112–176)	165 (89–180)	167 (110–187)	0.04
Anti-CCP (U/ml)	129 (67–204)	148 (98–204)	175 (85–285)	205 (159–246)	0.89
TNF-α (pg/ml)	26 (15–35)	30 (12–35)	35 (25–43)	43 (26–49)	0.33
IL-1β (pg/ml)	29 (22–45)	35 (23–42)	38 (24–42)	45 (27–50)	0.02
MiR-210	1.2 (1.15–1.67)	1.0 (0.89–1.17)	0.97 (0.89–1.19)	0.75 (0.65–0.85)	<0.001
MiR-155	3.9 (1.07–5.3)	5.8 (4.0–7.2)	6.1 (3.9–7.7)	9.0 (7.6–9.8)	<0.001

Note: P value calculated using contrast hypothesis test. Data presented as median (IQR). See Table 1 for abbreviations.

RA patients compared to healthy controls. These results are in line with reports that miR-210 may be associated with osteoarthritis (OA) [31,32]. In articular cartilage of OA rats, Zhang et al. [22] analysed miR-210 expression and showed that it was much lower than that of normal rats. Regarding miR-155, several studies have focused on its role in autoimmune diseases, such as RA. Stanczyk et al. [14] were first to report increased miR-155 expression in RA synovial fibroblasts compared to osteoarthritis synovial fibroblasts. Others observed that miR-155 expression is increased in RA peripheral blood mononuclear cells compared to normal controls [15,33].

Our multivariate regression analysis revealed that miR-210 and miR-155 are independent factors for detecting RA and can diagnose RA more accurately than existing laboratory markers. We also observed that low levels of miR-210 and increased miR-155 were associated with increased levels of TNF-α and IL-1β. This supports data from Qi et al. [34] who reported that overexpression of miR-210 inhibits expression of proinflammatory cytokines (IL-6 and TNF-α) induced by TLR4 in murine macrophages. They also noted that MiR-210 has anti-inflammatory and anti-apoptotic effects in LPS-induced chondrocytes. Moreover, our data extend that of Li et al. [33], who demonstrated that miR-155 in peripheral blood mononuclear cells of 45 RA patients was associated positively with increased TNF-α, IL-1β, CRP, RF, ESR, SJC, TJC and DAS-28. We predict that the down regulation of miR-210 and the upregulation of miR-155 are potential markers of RA disease activity and may have an important role in the pathogenesis of the disease. When analysing the influence of treatment on miR-210 and miR-155 expression, there was no significant difference between patients treated with DMARDs or biologics. This might be due to the small sample size in our study. Therefore, we recommend our data be confirmed in a larger population.

The major question now is how serum miR-210 and miR-155 relate to RA. Recently, some miRNAs have emerged to play an important role in the control of toll-like receptor (TLR) pathways [35]. These pathways can activate NF-κB and induce the expression of many genes responsible for the inflammatory response in OA and for the production of the proinflammatory cytokines. MiR-210 could regulate cell-cycle E2F3, cell differentiation, proliferation and apoptosis. Moreover, it stimulates the proliferation of fibroblast growth factor receptor protein 1 and homeobox A1 [36]. MiR-155 could increase TNF-α levels directly by binding to its 3′UTR or targeting gene transcripts coding for the repressor of TNF-α translation [37]. In germinal centres, miR-155 plays an important role in the development of B cells and mediates regulatory roles in T-cell homeostasis [38]. Moreover, miR-155 can contribute to RA pathogenesis by inducing chemokine production and downregulating chemokine receptor, which may lead to monocyte retention at the sites of inflammation in RA [39].

In conclusion, low levels of MiR-210 and high miR-155 may be useful markers of RA disease activity. Due to their correlations with serum TNF-α and IL-1β, both serum miR-210 and miR-155 may be implicated in the pathogenesis of the disease. This work represents an advance in biomedical science because it shows that serum miR-210 and miR-155 levels independently differentiate RA patients and healthy controls and are better than existing biomarkers for the diagnosis of RA.

Summary table

What is known about this subject

- Several miRNAs have been identified to play important roles in the regulation and the development of rheumatoid arthritis (RA)
- In contrast to cellular or tissue miRNAs, the expression profile of circulatory miRNAs in RA has not been fully investigated
- The role of serum MiR-210 and miR-155 in the diagnosis of RA has not been established yet

What this paper adds

- Downregulation of serum miR-210 and upregulation of miR-155 expression serve as non-invasive independent biomarkers for the diagnosis of RA
- *Serum miR-210 and miR-155 are potential markers of RA disease activity and may be implicated in the pathogenesis of the disease*

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet. 2001;358:903–911.
[2] Goekoop-Ruiterman YP, De Vries-Bouwstra JK, Allaart CF, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum. 2005;52:3381–3390.
[3] Breedveld FC, Weisman MH, Kavanaugh AF, et al. A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum. 2006;54:26–37.
[4] Choy E. Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology. 2012;51:v3–v11.
[5] Sun B, Liu X, Gao Y, et al. Downregulation of miR-124 predicts poor prognosis in pancreatic ductal adenocarcinoma patients. Br J Biomed Sci. 2016;73(4):152–157.
[6] Yadegaria ZS, Akramia H, Hosseinib SY, et al. miR-146a gene polymorphism and susceptibility to gastric cancer. Br J Biomed Sci. 2016;73(4):201–203.
[7] Ren X, Shen Y, Zheng S, et al. miR-21 predicts poor prognosis in patients with Osteosarcoma. Br J Biomed Sci. 2016;73(4):158–162.

[8] Liu C, Xing M, Wang L, et al. miR-199a-3p downregulation in thyroid tissues is associated with invasion and metastasis of papillary thyroid carcinoma. Br J Biomed Sci. 2017;74(2):90–94.
[9] Brooks WH, Le Dantec C, Pers JO, et al. Epigenetics and autoimmunity. J Autoimmun. 2010;34:J207–J219.
[10] Furer V, Greenberg JD, Attur M, et al. The role of microRNA in rheumatoid arthritis and other autoimmune diseases. Clin Immunol. 2010;136:1–15.
[11] Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Nat Acad Sci. 2008;105:10513–10518.
[12] D'Alessandra Y, Devanna P, Limana F, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur Heart J. 2010;31:2765–2773.
[13] Ji F, Yang B, Peng X, et al. Circulating microRNAs in hepatitis B virus-infected patients. J Viral Hepat. 2011;18:e242–e251.
[14] Stanczyk J, Pedrioli DM, Brentano F, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum. 2008;58(4):1001–1009.
[15] Pauley KM, Satoh M, Chan AL, et al. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Therapy. 2008;10(4):R101.
[16] Nakamachi Y, Kawano S, Takenokuchi M, et al. MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Rheum. 2009;60(5):1294–1304.
[17] Niederer F, Trenkmann M, Ospelt C, et al. Down-regulation of microRNA-34a* in rheumatoid arthritis synovial fibroblasts promotes apoptosis resistance. Arthritis Rheuma. 2012;64(6):1771–1779.
[18] Dong L, Wang X, Tan J, et al. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. J Cell Mol Med. 2014;18:2213–2224.
[19] Lin J, Huo R, Xiao L, et al. A novel p53/microRNA-22/Cyr61 axis in synovial cells regulates inflammation in rheumatoid arthritis. Arthritis Rheumatol. 2014;66:49–59.
[20] Xu T, Huang C, Chen Z, et al. MicroRNA-323-3p: a new biomarker and potential therapeutic target for rheumatoid arthritis. Rheumatol Int. 2014;34:721–722.
[21] Olarerin-George AO, Anton L, Hwang YC, et al. A functional genomics screen for microRNA regulators of NF-kappaB signaling. BMC Biol. 2013;11:19–34.
[22] Zhang D, Cao X, Li J, et al. MiR-210 inhibits NF-κB signaling pathway by targeting DR6 in Osteoarthritis. Sci Rep. 2015;5:12775–12782.
[23] Kurowska-Stolarska M, Alivernini S, Ballantine LE, et al. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. Proc Nat Acad Sci. 2011;108:11193–11198.
[24] Arnett FC, Edworthy SM, Bloch DA, et al. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315–324.
[25] Arnett FC, Edworthy SM, Bloch DA, et al. Modified disease activity scores thatinclude twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995;38:44–48.
[26] Westergren A. Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Med Scand. 1921;54:247–282.
[27] Everitt BS. Contrasts and custom hypothesis. The Cambridge dictionary of statistics. 2nd ed. Cambridge: CUP; 2002;13:329–338. ISBN 0-521-81099-X.
[28] Mody GM. Reflections on rheumatoid arthritis in selected sub-Saharan African countries. East Afr Med J. 2009;86:201–203.
[29] Cobb BS, Hertweck A, Smith J, et al. A role for Dicer in immune regulation. J Exp Med. 2006;203:2519–2527.
[30] Li QJ, Chau J, Ebert PJ, et al. miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell. 2007;129:147–161.
[31] Yamasaki K, Nakasa T, Miyaki S, et al. Angiogenic microRNA-210 is present in cells surrounding osteonecrosis. J Orthop Res. 2012;30:1263–1270.
[32] Zhang Y, Fei M, Xue G, et al. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. J Cell Mol Med. 2012;16:249–259.
[33] Li X, Tian F, Wang F. Rheumatoid arthritis-associated MicroRNA-155 targets SOCS1 and upregulates TNF-α and IL-1β in PBMCs. Int J Mol Sci. 2013;14:23910–23921.
[34] Qi J, Qiao Y, Wang P, et al. microRNA-210 negatively regulates LPS-induced production of proinflammatory cytokines by targeting NF-κB1 in murine macrophages. FEBS Lett. 2012;586(8):1201–1207.
[35] O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol. 2011;11:163–175.
[36] Samaan S, Khella HW, Girgis A, et al. miR-210 is a prognostic marker in clear cell renal cell carcinoma. J Mol Diagn. 2015;17:136–144.
[37] Faraonia I, Antonettib FR, Cardonea J, et al. miR-155 gene: a typical multifunctional. microRNA. Biochem Biophys Acta. 2009;1792(6): 497–505.
[38] Luzi E, Marini F, Sala SC, et al. Osteogenic differentiation of human adipose tissue derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Mineral Res. 2008;23(2):287–295.
[39] Elmesmari A, Fraser AR, Wood C, et al. MicroRNA-155 regulates monocyte chemokine and chemokine receptor expression in Rheumatoid Arthritis. Rheumatology. 2016;55(11):2056–2065.