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Serum LINC00305 expression and its genetic variant rs2850711 are associated with clinical and laboratory features of rheumatoid arthritis

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ABSTRACT

Background: Long intergenic non-protein coding (lnc) RNA 00305 (LINC00305) is a pro-inflammatory atherosclerosis-associated lncRNA. We hypothesised that LINC00305 expression and its variant rs2850711 (A/T) are implicated in rheumatoid arthritis (RA) and linked with clinical and routine laboratory markers.

Methods: 100 RA patients and 100 healthy controls were recruited. LINC00305 genotyping and expression were performed using allelic-discrimination PCR and quantitative real-time PCR. LINC00305 diagnostic power was evaluated using area under the receiver operating characteristic curve (AUC). Serum nuclear factor- κB (NF-κB) and matrix metalloproteinase-3 (MMP-3) levels were determined by ELISA, standard laboratory markers by routine methods.

Results: LINC00305 expression was significantly increased in RA patients and positively correlated with DAS28, C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor and anti-cyclic citrullinated peptide antibody. In multivariate analysis, NF-κB, MMP-3 and LINC00305 were significant predictors of RA ($P< 0.0001$). Individuals carrying AT and TT genotypes of rs2850711 polymorphism had significantly more likely to have RA than AA genotype carriers ($P< 0.05$). LINC00305 expression, DAS28 score and serum levels of NF-κB and MMP-3 were significantly increased in the patients carrying LINC00305 AT and TT genotypes as compared with AA genotype patients ($P< 0.01$).

Conclusion: Increased expression level of LINC00305 and its rs2850711 genetic variant may play a role in the diagnosis and management of RA, and its severity and activity.

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Introduction

Long noncoding RNAs (lncRNAs) are a group of RNA molecules 200 nucleotides or more in length, with effects on both the innate and the adaptive immune systems [1,2]. They may also be present in the circulation, so that detection in serum/plasma can provide early diagnostic and/or prognostic biomarkers for various diseases [3]. Previous studies reported the involvement of lncRNA in cancer development and pathogenesis of many diseases, and lncRNAs can regulate cytokines expression at the transcriptional level in cells of innate immune system in the context of autoimmune diseases [4]. However, their role in autoimmune diseases such as rheumatoid arthritis (RA) needs further investigation to validate the clinical utility of lncRNAs as diagnostic and therapeutic targets for these diseases.

RA is a chronic debilitating autoimmune inflammatory disease that is triggered by a blend of genetic, environmental and epigenetic factors. It primarily involves the lining of the synovial joints and is characterized by inflammatory immune cell infiltration of the synovium which is facilitated by synovial angiogenesis [5,6]. One of the many inflammatory mediators in RA is nuclear factor-κB (NF-κB) that acts on inducible transcription factors controlling multiple genes that play a role in cellular immune and inflammatory processes [7]. NF-κB signalling is highlighted as having lncRNAs involved in its regulation [8]. NF-κB activation plays a major role in the destructive and invasive potential of RA fibroblast-like synoviocytes (FLSs). In addition, NF-κB mediates transcriptional activation and induces expression of several matrix metalloproteinases (MMPs). MMPs are zinc-dependent endopeptidases which mediate basement membrane and extracellular matrix protein degradation, promoting tissue damage. Thus, their enhanced production in RA contributes to loss of cartilage and joint integrity [9]. Cells and cytokines involved in RA are also associated with the development and progression of atherosclerosis [10].

The lncRNA 00305 (LINC00305) promotes monocyte activation and production of inflammatory cytokines via the aryl hydrocarbon receptor repressor (AHRR)-NF-κB pathway in monocytes. LINC00305 is overexpressed in atherosclerotic plaques, and an atherosclerosis-associated single-nucleotide polymorphism (SNP) rs2850711, an A/T single-nucleotide variation located within the first intron of *LINC00305*, has been reported [11].

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We hypothesised altered expression of LINC00305 profiles in RA patients and linked with relevant clinical features and research and routine laboratory markers.

Subjects and methods

We tested our hypothesis on 100 RA patients (38 males, 62 females) and an equal number of control subjects (49 males, 51 females) ($p = 0.12$). Mean [SD] age of RA patients was 44.6 [8.4] and that for controls was 41.9 [11.9] ($p = 0.07$). The control subjects had no history of RA, or any other autoimmune or chronic diseases. Sample size was calculated using Daniel's formula [12]. Patients (diagnosed according to the ACR/EULAR classification 2010 criteria with score ≥6 points [13]) were recruited from outpatient Rheumatology and Orthopaedic Clinics in Suez Canal University Hospital, Ismailia, Egypt, from January 2018 to February 2019. Clinical examination and disease severity assessment using Disease Activity Score in 28 joints (DAS28) with C-reactive protein (CRP) were done for all RA patients [14]. Characteristics of RA patients are shown in Table 1. An informed consent was obtained from all patients prior to sample collection. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Faculty of Pharmacy, Suez Canal University (201709PHH1).

After overnight fasting, a 6 ml blood sample was drawn from each participant, from which 2 ml were collected in EDTA tubes for DNA extraction, 4 ml were collected in plain tubes for serum separation. Serum levels of CRP were measured using the latex agglutination slide test, erythrocyte sedimentation rate (ESR) was measured using the Westergren method, rheumatoid factor (RF) was evaluated using automated chemistry method (Cobas 6000), and Anti-cyclic citrullinated peptide (Anti-CCP) antibody was estimated by ELISA (ORG 601 Anti-CCP high sensitive). Any control subject with abnormal levels of any of these parameters was excluded from the study. RF values of ≥20 IU/mL were considered positive [15]. Anti-CCP values of ≥20 U/mL were considered positive. Serum levels of NF-κB and MMP-3 were measured using the human specific ELISA kits (BioSource International, CA, USA)

Total RNA was extracted from serum using miRNeasy Mini kit Isolation kit (Qiagen, Hilden, Germany). Concentration of RNA was determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Tech., Wilmington, DE, USA). Samples with a 260/280 nm absorbance ratio less than 1.8 were excluded. LINC00305 serum expression was quantitated using GoTaq® 1-Step RT-qPCR System (Promega, Madison, USA) and the StepOnePlus™ Real-Time PCR thermal cycling instrument (Applied Biosystems, Massachusetts, USA). Beta-actin (ß-actin) served as reference control for normalization. LINC00305 primer sequences were as following: Forward, 5′- TCAGCAGCCTTCTGGTTTATCA –3′; Reverse, 5′- TCCTTGCTTCCTTCAGGTCTCT –3′, while ß-actin primer sequences were: Forward, 5′-CACCCAGCACAATGAAGATC –3′; Reverse, 5′-GTCATAGTCCGCCTAGAAGC –3′. Annealing temperatures were 50°C and 56°C, respectively. The reaction volume was 20 µL containing 4 µL of RNA template, 1 µL of each of the two primers, 0.4 µL of GoScript™ RT mix for 1-step RT-qPCR, 10 µL of GoTaq® qPCR master mix, 0.31 µL of supplemental CXR reference dye, and 3.29 µL of nuclease-free water. The reactions were run in duplicate according to the following thermal profile: reverse transcription at 37°C for 15 min, then reverse transcriptase inactivation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing for 30 s, and extension at 72°C for 30 s. The fold change of LINC00305 expression in each patient was calculated based on the comparative cycle threshold method (ΔΔCT method); $2^{-\Delta\Delta CT}$ [16].

Genomic DNA was purified from whole blood using the Wizard genomic DNA purification kit (Promega, Madison, USA). Extracted DNA purity and concentration were assessed by NanoDrop ND-1000 (NanoDrop Technologies. Wilmington, DE, USA). LINC00305 rs2850711 genotyping was performed using Real-Time polymerase chain reaction (RT-PCR) allelic discrimination technology. PCR reactions were run blindly in duplicates in a 20-µL final volume containing 20 ng genomic DNA diluted to 9.5 µL with DNase-RNase-free water, 10 µL Taqman Universal PCR Master Mix, No AmpErase UNG (2x) and 0.5 µL 20 × TaqMan SNP Genotyping Assay Mix (Applied Biosystems, USA, assay ID C_16129208_10) with 100% concordance rate for genotype calls. Appropriate controls were used in each run. PCR amplification was performed on AB 7500HT analyser (Applied Biosystems, USA). PCR conditions started by initial denaturation at 95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min. Allelic discrimination was called by the SDS software version 2.1.1 (Applied Biosystems).

Table 1. Demographic and clinical characteristics of RA patients

Variables	RA patients (n = 100)	Reference range
Age of onset of disease (years)	38.2 [9.8]	-
DAS-28	5.2 [1.2]	0
ESR (mm/hr)	53 [29]	≤20 mm/hr
CRP (mg/L)	16 (8 – 30)	< 3.0 mg/L
RF (IU/ml)	44 (28–76)	(0–20 IU/ml)
RF positive cases	74	
RF negative cases	26	
Anti-CCP ab (U/ml)	41.2 (16.8–65.3)	(0–20 U/ml)
Anti-CCP ab positive cases	86	
Anti-CCP ab negative cases	14	

Data mean (SD), median (IQR), or number of subjects; DAS-28: 28-joints disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; CCP: cyclic citrullinated peptide.

Data analysis was performed using Graphpad prism software v.5 and SPSS version 17. Quantitative variables were expressed as mean with standard deviation (SD) and categorical variables were shown as frequencies and compared using the chi-square (χ2). Odds ratios (OR) with a 95% confidence interval (CI) were calculated. Hardy-Weinberg equilibrium was examined by a goodness-of-fit χ2 test. LINC00305, CRP, RF and anti-CCP antibodies levels were described by their median and interquartile ranges (IQR) as they did not fit a Gaussian distribution, differences detected by the Mann–Whitney U test for two independent groups. Multivariate analysis was used to evaluate the predictive variables associated with RA, including NF-κB, MMP-3 and LINC00305 expression. The diagnostic power for LINC00305 was estimated using the area under the receiver operating characteristic (AUC) curve. Based on the receiver operating characteristic analysis (ROC), the best cut-off point was selected and specificity and sensitivity were determined. Correlation analysis using Pearson's (r) and Spearman's rank (r_s) correlations was performed. The probability value ($P< 0.05$) was considered statistically significant.

Results

As shown in Table 2, there were significant increases in NF-κB and MMP-3 serum levels of RA patients when compared with the healthy controls. Furthermore, serum levels of LINC00305 in RA patients were significantly higher compared to the healthy control group. In multivariate analysis, NF-κB, MMP-3 and LINC00305 were significant predictors of RA. To assess whether LINC00305 can be used as diagnostic biomarker for RA, we measured the sensitivity and specificity using ROC curve analysis. The area under the ROC curves (95% CI) of LINC00305 for discriminating RA patients was 0.89 (0.80–1.00), $P < 0.0001$. At the optimal cut-off value of 1.5 fold change, sensitivity was 89% and specificity was 100%.

The associations of NF-κB, MMP-3 and LINC00305 levels with laboratory parameters of RA patients were also analysed. These levels were significantly positively correlated with DAS-28, ESR, CRP, RF and anti-CCP antibodies. LINC00305 expression levels exhibited a significant positive correlation with serum NF-κB and MMP-3 in patients with RA (Table 3).

As shown in Table 4, the minor T allele of rs2850711 (A/T) SNP within an intronic sequence of LINC00305 gene was more frequent in the RA patients (13.5%) than the control group (7%) and the major A allele was more common in control group (93%) than in the patients (86.5%) (odds ratio [95% confidence interval] for RA brought by T allele 0.49 [0.25–0.96]). Similarly, the AT genotype was linked to a reduced likelihood of RA compared to the AA genotype (0.46 [0.21–0.98]), whilst the AT+TT genotype was linked to a reduced likelihood of RA (0.47 [0.23–0.99]). Genotypes distribution was compatible with Hardy–Weinberg equilibrium in the whole study sample ($P= 0.92$). Patients carrying AT+TT genotypes had higher DAS-28, ESR, CRP and anti-CCP antibodies than AA homozygote patients. Moreover, serum NF-κB and MMP-3 levels were significantly higher in the carriers of the T allele (AT + TT genotypes) compared to the AA genotype carriers.

Discussion

RA is an autoimmune inflammatory disease characterized by immune cells infiltration into the synovial compartment. Initiation of chronic inflammation is attributable to development of an autoimmune response through immune cells interaction leading to NF-κB activation, production of inflammatory cytokines in RA synovium and consequently progression to a sustained, self-perpetuated inflammation [17]. Our data confirm numerous reports of increased NF-κB in RA [18]. We reasoned that as inflammation is the main attribute of RA, but less so in atherosclerosis [10] and that LINC00305 was previously identified as a pro-inflammatory lncRNA with increased expression in atherosclerotic plaques and monocytes [11], the lncRNAs may also be altered in RA. Our results revealed that the expression of LINC00305 was indeed increased in serum samples of RA patients and was positively correlated with DAS-28 score and several routine markers, making LINC00305 a potential prognostic biomarker and therapeutic target in RA with a very significant area under the ROC curve.

Previous studies have shown that LINC00305 modulates NF-κB activity by binding to lipocalin-1 interacting membrane receptor (LIMR), promoting the interaction between LIMR and aryl-hydrocarbon receptor repressor (AHRR), the repressor of AHR, leading to enhanced expression and nuclear localization of AHRR which positively regulates NF-κB activity and revealed that LINC00305 overexpression promotes the expression of inflammation-associated genes [11]. LINC00305

Table 2. Multivariate analysis of factors associated with RA

Variables	Data	Odds ratio (95% CI)
NF-κB (ng/ml)		
Controls	0.8 [0.1]	3.21
Patients	4.0 [0.8]	(3.06–3.36)
MMP-3 (ng/ml)	0.4 [0.1]	2.97
Controls		(2.82–3.11)
Patients	3.5 [0.7]	
LncRNA LINC00305		
Controls	1	5.74
Patients	7.3 (5.9–8.6)	(5.19–6.30)

Data mean (SD) or median (IQR). CI: confidence interval; OR: odds ratio; RA: rheumatoid arthritis; NF-κB: nuclear factor-kappa beta; MMP-3: matrix metalloproteinase-3. All differences $P < 0.0001$.

Table 3. The association of NF-κB, MMP-3 and LINC00305 levels with disease biomarkers in RA patients

	Clinical characteristics and biomarkers						
Disease biomarkers	DAS-28	ESR	CRP	RF	Anti-CCP ab	NF-κB	MMP-3
NF-κB	r = 0.84	r = 0.85	r_s = 0.86	r_s = 0.71	r_s = 0.73	– – –	– – –
MMP-3	r = 0.77	r = 0.76	r_s = 0.92	r_s = 0.68	r_s = 0.74	– – –	– – –
LINC00305	r_s = 0.89	r_s = 0.91	r_s = 0.91	r_s = 0.67	r_s = 0.75	r_s = 0.84	r_s = 0.91

(r): Pearson's correlation coefficient; (r_s): Spearman's correlation coefficient; RA: rheumatoid arthritis; DAS-28: 28-joints disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; CCP: cyclic citrullinated peptide; NF-κB: nuclear factor-kappa beta; MMP-3: matrix metalloproteinase-3.* All correlations significant at $P < 0.0001$

Table 4. LINC00305 genotypes and their association with disease biomarkers.

Variables		Patients/ Controls (n)	*P value*
LINC00305 alleles	A	175/186	0.035
	T	27/14	
LINC00305 genotypes	AA	76/87	
	AT	23/12	0.04
	TT	1/1	
		Patients	
DAS-28	AA	5.0 [1.2]	
	AT+ TT	5.7 [1.0]	0.005
ESR (mm/hr)	AA	50 [28]	
	AT+ TT	66 [30]	0.015
NF-κB (ng/ml)	AA	3.8 [0.7]	
	AT+ TT	4.4 [0.7]	0.0017
MMP-3 (pg/ml)	AA	3.3 [0.7]	
	AT+ TT	3.8 [0.8]	0.0013
CRP (mg/L)	AA	15 (8–27)	
	AT+ TT	25 (12–45)	0.0148
RF (IU/ml)	AA	39 (19–57)	
	AT+ TT	72 (43–121)	0.0022
Anti-CCP ab (U/ml)	AA	34 (13–57)	
	AT+ TT	63 (37–102)	0.0074
LINC00305 fold change	AA	6.9 (5.0–8.2)	
	AT+ TT	8.1 (6.9–10.3)	0.0033

AA n = 74, AT+TT n = 24. Data mean [SD] or median [IQR]. Abbreviations as table 3.

activates the Notch/NF-κB pathway by down-regulating miR-124 [19], whose levels were previously identified to be decreased in RA synoviocytes [20]. Furthermore, miR-124 directly binds to 3′- UTR of subunit of NF-κB p65 inhibiting its expression, thus suppressing pro-inflammatory cytokine production [21]. Notably, therefore, our results extend this link, revealing a significant positive correlation between LINC00305 and serum NF-κB levels (Figure 1).

A key aspect of RA is that fibroblast-like synoviocytes (FLSs) become hyperplastic and invasive, leading to cartilage and bone destruction [5]. Thus, proteolytic pathways play a crucial role in RA development and progression. FLSs express many NF-κB-induced genes and secrete MMPs which are able to cause destruction of hyaline articular cartilage [22]. Increased expression of MMP-3 may play a role in collagen degradation in RA [23]. Consistent with these findings, our results showed a significant increase in serum MMP-3 levels in RA patients. Also, there was a significant positive correlation between LINC00305 expression and serum MMP3 levels. LINC00305 may affect MMP-3 expression through NF-κB activation which mediates transcriptional activation of several genes including MMPs [24]. In addition, LINC00305 induced miR-124 down-regulation may enhance the proliferation and invasion of RA synovial fibroblasts through up-regulating its target proteins including the inhibitory member of the apoptosis stimulating protein of p53 (iASPP), which is a key inhibitor of tumour suppressor p53 [25,26]. iASPP up-regulation has a confirmed pathogenic role in RA, reduces the expression of p21, a cell cycle inhibitor regulated by p53, leading to activation of activator protein-1 (AP-1) which plays a role in the regulation of MMP secretion in RA FLSs [27,28]. There are several other pathways linking MMPs to NF-κB, including monocyte chemoattractant protein 1 (MCP-1), and the extracellular signal-regulated kinase 1/2 and P38 mitogen-activated protein kinase pathways [29–31]. Also, activation increases the expression of iASPP through p65/p50 binding to a putative NF-κB-binding site in the iASPP promoter [32] and regulates transcription of MCP-1 gene [33] (Figure 1).

Previous research found that lncRNA LINC00305 associated SNP rs2850711 influences the inflammatory environment in atherosclerosis [11]. We found that the minor T allele was more prevalent among

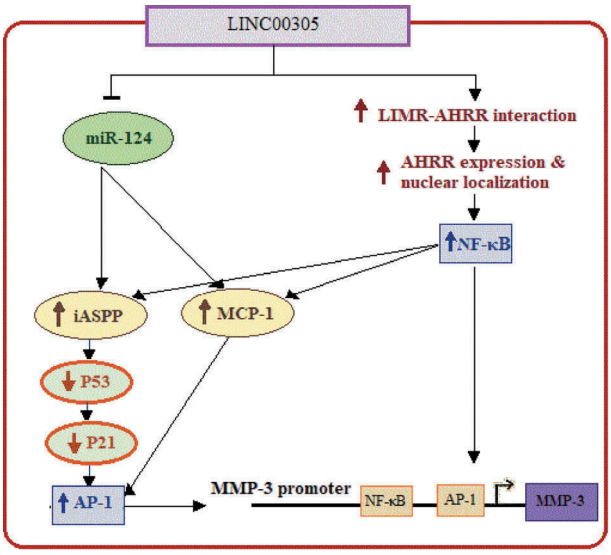

Figure 1. Potential role of LINC00305 in the regulation of MMP-3.
Note: Schematic of possible interactions in the signal transduction pathways promoting MMP-3 expression

RA patients, and patients carrying the T allele as either homozygote (TT) or heterozygote (AT) showed a significantly increased DAS-28 score and relative expression of lncRNA LINC00305 compared to the AA homozygotes. Furthermore, LINC00305 rs2850711 was linked with increased severity and activity of RA. Accordingly, we provide novel insights on the role of LINC00305 and its associated SNP in pathogenetic mechanisms involved in RA that may contribute to advances in RA diagnosis and therapy. The current study was limited by the relatively small sample size. We recommend further studies at a larger scale on different populations and further functional evaluation of this polymorphism and its target lncRNA, as this might be an interesting target for future therapeutic interventions in RA patients.

This study represents an advance in biomedical science because it provides novel aspects of the role of LINC00305 and its variant in RA pathogenesis and may contribute to advances in RA diagnosis and therapy.

Summary table

What is known about this subject:
- LINC00305 is a pro-inflammatory atherosclerosis-associated lncRNA
- Atherosclerosis has an inflammatory component

What this paper adds:
- RA is associated with increased LINC00305
- LINC00305 SNP rs2850711 is associated with MMP-3, Nf-kB, routine markers and increased severity and activity of RA

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Disclosure statement

The authors declare no conflicts of interest.

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References

[1] Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A. 2009;106:11667–11672.
[2] Theofilopoulos AN, Kono DH, Baccala R. The multiple pathways to autoimmunity. Nat Immunol. 2017;18:716–724.
[3] Jiang X, Lei R, Ning Q. Circulating long noncoding RNAs as novel biomarkers of human diseases. Biomark Med. 2016;10:757–769.
[4] Müller N, Döring F, Klapper M, et al. Interleukin-6 and Tumour Necrosis Factor-α differentially regulate lincRNA transcripts in cells of the innate immune system in vivo in human subjects with rheumatoid arthritis. Cytokine. 2014;68:65–68.
[5] Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res. 2002;4:S81- S90.
[6] Guo Q, Wang Y, Xu D, et al. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res. 2018;6:15.
[7] Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023.
[8] Pearson MJ, Jones SW. Long non-coding RNAs in the regulation of inflammatory pathways in rheumatoid arthritis and osteoarthritis. Arthritis Rheumatol. 2016;68:2575–2583.
[9] Ni S, Li C, Xu N, et al. Follistatin-like protein 1 induction of matrix metalloproteinase 1, 3 and 13 gene expression in rheumatoid arthritis synoviocytes requires MAPK, JAK/STAT3 and NF-κB pathways. J Cell Physiol. 2019;234:454–463.
[10] Skeoch S, Bruce IN. Atherosclerosis in rheumatoid arthritis: is it all about inflammation? Nat Rev Rheumatol. 2015;11:390–400.
[11] Zhang -D-D, Wang W-T, Xiong J, et al. Long noncoding RNA LINC00305 promotes inflammation by activating the AHRR-NF-κB pathway in human monocytes. Sci Rep. 2017;7:46204.
[12] Naing L, Winn T, Rusli B. Practical issues in calculating the sample size for prevalence studies. Arch Orofac Sci. 2006;1:9–14.
[13] Aletaha D, Neogi T, Silman AJ, et al. rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;20 10:2569–2581.
[14] van der Heijde DM, van 'T Hof MA, van Riel PL, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis. 1990;49:916–920.
[15] Knijff-Dutmer E, Drossaers-Bakker W, Verhoeven A, et al. Rheumatoid factor measured by fluoroimmunoassay: a responsive measure of rheumatoid arthritis disease activity that is associated with joint damage. Ann Rheum Dis. 2002;61:603–607.
[16] Kenneth J, Livak TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–408.
[17] Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. Immunity. 2014;41:694–707.
[18] Makarov SS. NF-κB in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. Arthritis Res. 2001;3:200–206.
[19] Li X, Yu M, Han L, et al. LINC00305 represses miR-124 expression to trigger inflammatory insults in the

presence of lipopolysaccharide. Artif Cells Nanomed Biotechnol. 2019;47:2352–2360.
[20] Ceribelli A, Yao B, Dominguez-Gutierrez PR, et al. MicroRNAs in systemic rheumatic diseases. Arthritis Res Ther. 2011;13:229.
[21] Qiu S, Feng Y, LeSage G, et al. Chronic morphine-induced microRNA-124 promotes microglial immunosuppression by modulating P65 and TRAF6. J Immunol. 2015;194:1021–1030.
[22] Sabeh F, Fox D, Weiss SJ. Membrane-type I matrix metalloproteinase-dependent regulation of rheumatoid arthritis synoviocyte function. J Immunol. 2010;184:6396–6406.
[23] Ainola M, Mandelin J, Liljeström MP, et al. Pannus invasion and cartilage degradation in rheumatoid arthritis: involvement of MMP-3 and interleukin-1b. Clin Exp Rheumatol. 2005;23:644–650.
[24] Okamoto T, Sanda T, Asamitsu K. NF-κB signaling and carcinogenesis. Curr Pharm Des. 2007;13:447–462.
[25] Li J, Song Q, Shao L, et al. MiR-124a inhibits proliferation and invasion of rheumatoid arthritis synovial fibroblasts. Eur Rev Med Pharmacol Sci. 2018;22:4581–4588.
[26] Dong P, Ihira K, Hamada J, et al. Reactivating p53 functions by suppressing its novel inhibitor iASPP: a potential therapeutic opportunity in p53 wild-type tumors. Oncotarget. 2015;6:19968–19975.
[27] Chen S-Y, Shiau A-L, Wu C-L, et al. P53-derived hybrid peptides induce apoptosis of synovial fibroblasts in the rheumatoid joint. Oncotarget. 2017;8:115413–115419.
[28] Zeisel MB, Druet VA, Wachsmann D, et al. MMP-3 expression and release by rheumatoid arthritis fibroblast-like synoviocytes induced with a bacterial ligand of integrin α5β1. Arthritis Res Ther. 2004;7:R118–R126.
[29] 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;60:12 94–1304.
[30] Yang C-Q, Li W, Li S-Q, et al. MCP-1 stimulates MMP-9 expression via ERK 1/2 and p38 MAPK signaling pathways in human aortic smooth muscle cells. Cell Physiol Biochem. 2014;34:266–276.
[31] Viedt C, Vogel J, Athanasiou T, et al. Monocyte chemoattractant protein-1 induces proliferation and interleukin-6 production in human smooth muscle cells by differential activation of nuclear factor-κB and activator protein-1. Arterioscler Thromb Vasc Biol. 2002;22:914–920.
[32] Lu B, Guo H, Zhao J, et al. Increased expression of iASPP, regulated by hepatitis B virus X protein-mediated NF-κB activation, in hepatocellular carcinoma. Gastroenterology. 2010;139:2183–2194.e2185.
[33] Kumar Rajendran N, George BP, Chandran R, et al. The influence of light on reactive oxygen species and NF-κB in disease progression. Antioxidants. 2019;8:640.