Polymorphisms in microRNA binding site of *SET8* regulate the risk of rheumatoid arthritis

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Abstract. Rheumatoid arthritis (RA) is a complex, heterogeneous, progressive and long-term autoimmune disease characterized by symmetrical joint inflammation and bone erosion. The etiology of RA is unclear, but its pathogenesis is associated with oxidative stress and inflammatory cytokines. Single nucleotide polymorphisms (SNPs) in the microRNA (miRNA)-binding sites modify the development of rheumatic disease by regulating the expression of target genes. The present study investigated whether SNPs in the miRNA binding site in the 3' untranslated region (3'-UTR) of SET domain containing (lysine methyltransferase) 8 (*SET8*) and *Keratin 81* (*KRT81*), namely rs16917496 and rs3660, respectively, were associated with the occurrence of RA. The polymerase chain reaction-ligase detection reaction assay showed that the distribution frequencies of the CC genotype (P=0.025) of SNP rs16917496 in *SET8* were significantly higher in patients with RA than in healthy controls, which indicated that the CC genotype was associated with an increased risk of RA. *SET8* expression in the blood samples of CC genotype carriers was lower than that of TT genotype carriers. Moreover, the CC genotype carriers exhibited higher reactive oxygen species (ROS) levels (1011.500±536.426 vs. 548.616±190.508, P=0.032) and lower interleukin-10 (IL-10) levels (P<0.001). The present study demonstrated that SNP rs16917496 in the 3'-UTR of *SET8* was a predictor of RA risk and may regulate RA development by mediating expression of *SET8*, thereby regulating the levels of ROS and IL-10.

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Introduction

Rheumatoid arthritis (RA) is a complex, heterogeneous, progressive and long-term autoimmune disease characterized by symmetrical joint inflammation and bone erosion (1). The global prevalence of RA is 0.3-1.0% in females, which is five times higher than that in males (2). The risk factors for RA include genetic susceptibility, infectious agents, oxidative stress and inflammatory cytokines. RA is characterized by extensive infiltration of immune cells in the synovium, synovial hyperplasia, joint inflammation and excessive pro-inflammatory cytokine production. Oxidative stress caused by increased production of reactive oxygen species (ROS) and inflammatory cytokines contributes to the pathogenesis of RA (3-5).

MicroRNAs (miRNAs or miRs) are non-coding RNAs that are ~22 nucleotides long; they regulate the expression of target genes by binding to the 'seed region', 2-8 nucleotides of the 3' untranslated region (3'-UTR), as a post transcriptional regulator of messenger RNA (mRNA). The perfect complementarity between miRNAs and target mRNA sequences may lead to RNA silencing, resulting in reduced protein expression (6-9). A single nucleotide polymorphism (SNP) is a polymorphism of a DNA sequence caused by single nucleotide variation at the genome level. SNPs in the 3'-UTR of the target gene might affect its binding affinity to the corresponding miRNA, altering gene expression, which could change the biological functions of cells and initiate the onset of the diseases (10). The miRNA-related SNPs (miR-SNPs) rs2296135 and rs1131445 in interleukin-15 receptor α (*IL-15RA*) and IL-16 gene are associated with risk of RA (11,12).

SET domain containing (lysine methyltransferase) 8 (*SET8*), also known as *PR-SET domain-containing protein 7* (*PR-SET7*) or *Lysine methyltransferase 5a* (*KMT5a*), encodes histone H4 lysine 20 monomethyl transferase, which is involved in cell cycle, DNA damage repair and cancer development (13-16). *SET8* regulates the host immune response through the miR-30e-3p/FoxO3a/SET8 axis during mycobacterial infection (17). miR-502 regulates *SET8* expression via its binding site in the 3'-UTR of *SET8* mRNA and SNP rs16917496 at this site is associated with *SET8* expression (14-16). Keratin is involved in RA; therefore, anti-keratin antibodies are used for RA diagnosis (18). Keratin 81 (*KRT81*) is a member of the keratin gene family that encodes hair

keratin, which is a key component of the intermediate fila-ments and maintains mechanical stability and integrity of epithelial cells (19). Moreover, SNP rs3660 in the miRNA binding site of the 3'-UTR of *KRT81* mediates the expres-sion of *KRT81* (20,21). In previous studies, miR-SNPs have been associated with the risk of RA (11,12); therefore, the present case-control study aimed to assess the association of miR-SNPs with risk of RA.

Materials and methods

Blood collection and DNA extraction. A total of 2 ml blood samples were collected from 82 patients with RA from May to December 2017 at the Department of Immunology and Rheumatology of the Second Hospital of Hebei Medical University (Shijiazhuang, China). Blood samples were also collected from 105 sex- and age- matched healthy controls. The diagnostic standard for RA was based on the 2010 revised standard of the American College of Rheumatology. Inclusion criteria were patients diagnosed as RA who were between 18 and 80 years old (inclusive) who had not yet started drug therapy. Patients <18 or > 80 years old, pregnant and lactating patients and patients with infectious inflamma-tory diseases were excluded (22). The clinical characteristics of patients with RA, including age, sex, disease activity score (DAS28) and laboratory results, such as anti-cyclic citrul-linated peptide antibody (anti-CCP) and C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF) levels, were recorded. Disease activity score was based on DAS28: ≤2.6, clinical remission; >2.6 and ≤3.2, low activity; >3.2 and ≤5.1, moderate activity and >5.1, high activity (23). DNA from blood samples was extracted using a TIANamp Blood Clot DNA Kit (Tiangen) according to the manufacturer's instruction. All procedures were approved by the Human Tissue Research Committee of The Second Hospital of Hebei Medical University (approval no. 2017-P031). All participants provided written informed consent prior to enrollment.

PCR and sequence analysis. SNPs of the miRNA binding sites of *SET8* (rs16917496) and *KRT81* (rs3660) were geno-typed based on the sequences available in the National Center for biotechnology information SNP database (ncbi.nlm.nih. gov/snp/) using a PCR-ligase detection reaction assay that amplified the DNA fragment flanking the miR-SNPs. The probes used for miR-SNPs genotyping were as follows: Probe S1 and S2 located upstream of the SNPs matched alleles of the miR-SNPs with length difference of three base pairs and probe S3 located downstream of SNPs with the complemen-tary sequence. Following ligation with the PCR products using probes S1 + S3 or S2 + S3, SNPs were detected and verified based on length of ligated products. The sequences of primers and probes are listed in Table I. PCR was performed using a DreamTaq Green PCR Master Mix (2X) (K1081) (Shanghai Yisheng Biotechnology Co., Ltd.). The thermocycling condi-tions were as follows: 5 min denaturation at 95°C followed by 35 cycles of 20 sec denaturation at 94°C, 20 sec annealing at 55°C and 40 sec extension at 72°C and final extension at 72°C for 10 min. Ligation was performed using different probes and the ligated products were separated using an ABI PRISM

genetic analyzer 3730XL (Applied Biosystems; Thermo Fisher Scientific, Inc.). Polymorphisms were confirmed by repeating the analysis of two DNA strands.

Western blotting. Western blotting was performed to confirm the association between *SET8* expression and SNPs rs16917496. Total protein was isolated from human periph-eral blood mononuclear cells using a ReadyPrep protein extraction kit (Bio-Rad Laboratories, Inc.) according to the manufacturer's instructions. BCA assay was used to determine protein concentration. A total of 40 μg total protein/lane was loaded in a 10% denaturing polyacrylamide gel for separa-tion and transferred to polyvinylidene difluoride membranes. The membranes were blocked using 5% skimmed milk powder with TBST containing 0.1% Tween-20 for 1 h at room temperature, then incubated at 4°C overnight with the following primary antibodies: Mouse monoclonal anti-*SET8* (Abcam; cat. no. ab3798; 1:500) or anti-β-actin antibody (Santa Cruz Biotechnology, Inc.; cat. no. SC-47778A; 1:20,000). The membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (Thermo Fisher Scientific, Inc.; cat. no. 31430, 1:10,000) at room temperature for 2 h. FluorChem HD2 (ProteinSimple) was used to detect signals.

Determination of ROS levels. BBOXiProbe® serum active oxygen detection kit (BestBio Technology) was used to deter-mine total serum ROS levels. Briefly, blood was centrifuged at 1,000 x g for 5 min at 25°C to obtain serum, and 100 μl serum with 10 μl O12 probe were incubated at 37°C for 30 min. ROS levels were measured using a fluorescence microplate reader (BioTek Instruments, Inc.) at an excitation wavelength of 488 nm and an emission wavelength of 520 nm.

Determination of cytokine levels. Human TH1/TH2 panel (8-plex) in a filter plate V02 (BioLegend, Inc.) was used to measure the levels of IL-5, 13, 2, 6, 10 and 4, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). A total of 25 μl serum sample (two-fold diluted with assay buffer) was incu-bated for 30 min at 25°C with 25 μl Human Th Panel Detection antibody (BioLegend, Inc.; cat. no. 741041, stock solution), mixed with 25 μl Capture beads (BioLegend) for 1 h in the dark at room temperature and shaken using an oscillator at ~800 rpm for 1 h at 25°C. The sample was supplemented with 25 μl streptavidin-phycoerythrin and shaken using an oscil-lator at ~800 rpm in the dark for 30 min at room temperature. The PE fluorescence signal of the analyte-specific bead region was quantitatively analyzed using MACSQuant analyzer 10 (Miltenyi Biotec GmbH) and the analyte concentra-tion was determined using LEGENDplex™ software V8.0 (BioLegend, Inc.).

Statistical analysis. Unpaired student's t test was used for the analysis of continuous variables of independent samples. Data are presented as the mean and standard deviation. The independent experiments were repeated three times. Wilcoxon rank-sum test was used if the assumption of normality was incomplete. χ^2 or Fisher's exact test was used to evaluate categorical variables in the contingency tables. All statistical analysis was performed using SPSS software (version 25.0;

Table I. Primers and probes for microRNA-associated SNP genotyping.

Gene	SNP	Primer sequence	Probe sequence
SET8	rs16917496 (C/T)	F: 5'-TCAGCAAATTGGA AGAGGAT-3' R: 5'-GTGGCCTTGCCTG GCAAT-3'	S1: 5'-ctgactgaTTGTGGTTTAGCTTTGTATTTAAAC-3' S2: 5'-ctgactgactgTTGTGGTTTAGCTTTGTATTTAAAT-3' S3: 5'-AAGGAAATAAACTTGAAAATTATTTGTCATC-3'
KRT81	rs3660 (C/G)	F: 5'-GGGGATCACACAG AGAAATGT-3' R: 5'-ATCTTTCCTGCCC TGCCTTG-3'	S1: 5'-ctgactgactgaGAGTGGGAGGGGTCTTTCAAAGTGC-3' S2: 5'-ctgactgactgactgGAGTGGGAGGGGTCTTTCAAAGTGG-3' S3: 5'-AGGAGAAGTAGCTGAGCACTTGCTCctgactgac-3'

SNP, single nucleotide polymorphism; F, forward; R, reverse; SET, SET domain containing (lysine methyltransferase); KRT, keratin. Probe sequence, the upper case is the internal gene sequence, and the lower case is the sequence artificially added into 5' terminal of S1 and S2 probes to make the two probes different in length.

Table II. Clinical characteristics of patients with rheumatoid arthritis and controls.

Characteristic	Patients (n=82)	Controls (n=105)	t/χ^2	P-value
Mean age, years	53.71±11.59	52.02±8.49	1.11	0.27
Sex, male/female	16/66	24/81	0.31	0.58
Other immunological disease, yes/no	37/45	NA	NA	NA
Anti-CCP, +/-	53/29	NA	NA	NA
CRP, +/-	61/21	NA	NA	NA
ESR, +/-	70/12	NA	NA	NA
RF, +/-	61/21	NA	NA	NA
DAS28				
≤2.6	2	NA	NA	NA
2.6<DAS28≤3.2	3	NA	NA	NA
3.2<DAS28≤5.1	18	NA	NA	NA
>5.1	59	NA	NA	NA

NA, not applicable; CCP, cyclic citrullinated peptide; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; DAS28, disease activity score in 28 joints.

IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

SET8 genotype is associated with risk of RA. A total of 82 patients with RA were compared with 105 sex- and age-matched healthy controls. The clinical characteristics of patients and the control group are listed in Table II.

SNPs in the miRNA binding sites of *SET8* (rs16917496) and *KRT81* (rs3660) in patients with RA and healthy controls were genotyped to evaluate their impact on the risk of RA. There was no significant difference in the genotype distribution frequency of SNP rs3660 in *KRT81* ($P=0.091$). For SNP rs16917496 in *SET8*, the frequencies of genotypes CC and CT + TT were 10.98 and 89.02 in patients with RA and 2.86 and 97.14% in controls, respectively. The CC genotype was associated with a 4.192-fold increased risk of RA compared with that of CT + TT (odds ratio, 4.192; 95% CI: 1.097-16.021;
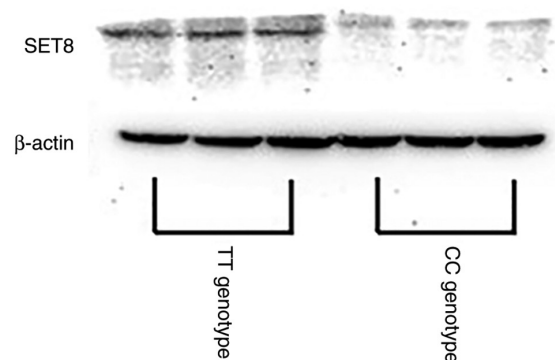

Figure 1. Expression of *SET8* protein in patients with TT and CC genotype of rheumatoid arthritis. *SET8*, SET domain containing (lysine methyltransferase) 8.

$P=0.025$; Table III). The CC genotype of *SET8* was also associated with lower protein expression than TT (Fig. 1). These

Table III. Frequency distribution of SNP sites in patients with rheumatoid arthritis and controls.

Gene (SNP)	Genotype	Patients (n=82)	Controls (n=105)	χ^2	P-value	OR	95%CI
SET8	CC	9	3	5.053	0.025	4.192	1.097-16.021
(rs16917496)	CT + TT	73	102				
KRT81	CC	56	59	2.848	0.091	1.679	0.918-3.072
(rs3660)	CG + GG	26	46				

SNP, single nucleotide polymorphism; *SET8*, SET domain containing (lysine methyltransferase) 8; *KRT81*, keratin 81.
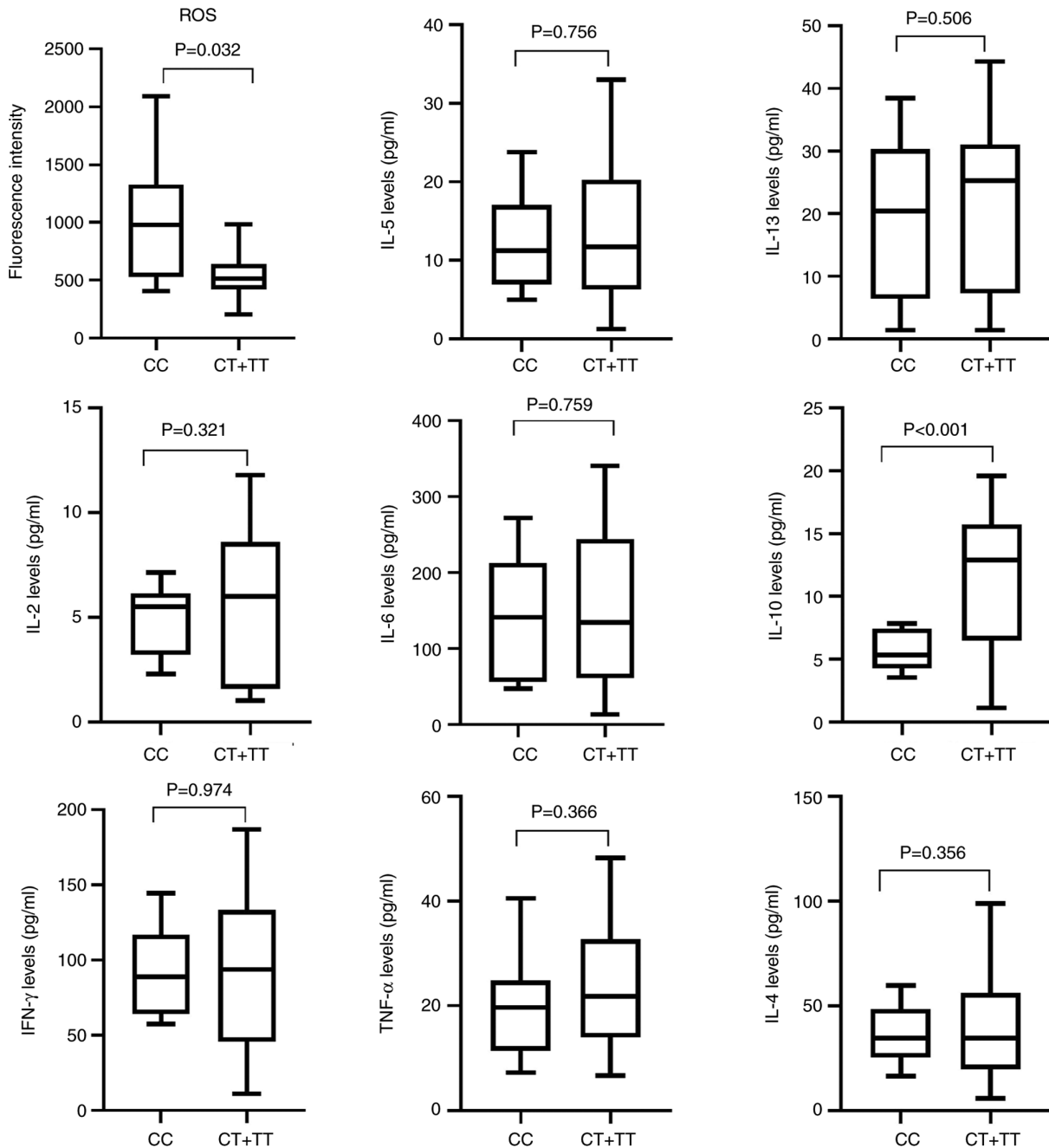

Figure 2. Association between rs16917496 genotype and ROS and cytokine levels. ROS, reactive oxygen species.

data implied that SNP rs16917496 was associated with *SET8* expression and regulated RA onset.

Association of SET8 genotype with ROS and cytokines levels. In previous studies, increased ROS levels have been reported

in patients with RA (24,25); therefore, the present study investigated the relationship between SNP rs16917496 and ROS levels in patients with RA. ROS levels were higher in the CC genotype carriers (1011.500±536.426 vs. 548.616±190.508, P=0.032, Fig. 2) than in the CT + TT genotype carriers. CC genotype was significantly associated with lower IL-10 levels (P<0.001; Fig. 2). These data suggested that SNPs regulated RA development by mediating ROS production and cytokine expression. There was no significant association between SNP rs16917496 and clinical characteristics of the participants, including age, sex, levels of anti-CCP and CRP, ESR, RF levels and DAS28 (data not shown).

Discussion

miRNAs serve important roles in the post-transcriptional regulation by controlling mRNA translation (26,27). SNPs in the miRNA binding sites can alter miRNA-mRNA binding affinity, which alters expression of the corresponding genes, thereby affecting cell proliferation, apoptosis and development. This process is associated with susceptibility to diseases, including cancer and autoimmune diseases (20,28,29). Here, SNP rs16917496 in the miR-502 binding site of the 3'-UTR of *SET8* mRNA was associated with the occurrence of RA; low *SET8* and higher ROS levels and decreased IL-10 expression were associated with CC genotype and increased risk of RA.

Consistent with the results of a previous study, the present study elucidated that the CC genotype of *SET8* was associated with low *SET8* expression (30). *SET8* overexpression inhibits high glucose-mediated ROS accumulation in human umbilical vein endothelial cells (31) and a decrease in *SET8* levels could enhance intracellular ROS levels. Oxidative stress is involved in the development of angiogenesis, synovial proliferation and inflammatory infiltration in RA (32,33). ROS can damage cartilage and extracellular matrix components by promoting the formation of rheumatoid factors and reducing the synthesis of collagen and proteoglycans in the pathogenesis of RA (34). Moreover, ROS oxidize lipids, nucleic acids and proteins to accelerate RA development (33). Therefore, functional analysis is required to assess the impact of enhanced ROS production induced by low *SET8* levels during RA onset.

IL-10 inhibits expression of inflammatory factors, such as TNF-α, IL-17 and IL-1 β via the NF-κB signaling pathway (35,36). Moreover, IL-10 overexpression in bone marrow mesenchymal stem cells significantly alleviates RA symptoms by decreasing proliferation of synovium and promotes the repair of articular cartilage in collagen-induced arthritis (CIA) rats (36). It also decreases synovial inflammation, bone destruction and articular cartilage damage in CIA rats (37). The underlying mechanisms for RA risk-associated SNPs and IL-10, and that between *SET8* and IL-10, should be evaluated.

The present findings suggested that the SNPs in the miR-502 binding site of the 3'-UTR of *SET8* mRNA were predictors of RA risk and may regulate RA pathogenesis by mediating expression of *SET8* to regulate ROS and IL-10 levels.

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Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SZ designed the experiments. YZ and ZS collected tissue specimens. XZ and JZ performed the experiments. CP and SZ interpreted the data and drafted the manuscript. SZ and CP confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study involving human participants was approved by The Human Tissue Research Committee at the Second Hospital of Hebei Medical University (approval no. 2017-P031).

Patient consent for publication

All subjects provided written informed consent to participate in this study.

Competing interests

The authors declare that they have no competing interests.

References

1. Behl T, Upadhyay T, Singh S, Chigurupati S, Alsubayiel AM, Mani V, Vargas-De-La-Cruz C, Uivarosan D, Bustea C, Sava C, *et al*: Polyphenols targeting MAPK mediated oxidative stress and inflammation in rheumatoid arthritis. Molecules 26: 6570, 2021.
2. Dar WR, Mir IA, Siddiq S, Nadeem M and Singh G: The assessment of fatigue in rheumatoid arthritis patients and its impact on their quality of life. Clin Pract 12: 591-598, 2022.
3. Alturaiki W, Alhamad A, Alturaiqy M, Mir SA, Iqbal D, Bin Dukhyil AA, Alaidarous M, Alshehri B, Alsagaby SA, Almalki SG, *et al*: Assessment of IL-1β, IL-6, TNF-α, IL-8, and CCL 5 levels in newly diagnosed Saudi patients with rheumatoid arthritis. Int J Rheum Dis 25: 1013-1019, 2022.
4. Wang X, Fan D, Cao X, Ye Q, Wang Q, Zhang M and Xiao C: The role of reactive oxygen species in the rheumatoid arthritis-associated synovial microenvironment. Antioxidants (Basel) 11: 1153, 2022.
5. Mateen S, Zafar A, Moin S, Khan AQ and Zubair S: Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. Clin Chim Acta 455: 161-171, 2016.
6. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.
7. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.
8. Zeng Y, Yi R and Cullen BR: MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci USA 100: 9779-9784, 2003.
9. Zeng Y, Wagner EJ and Cullen BR: Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. Mol Cell 9: 1327-1333, 2002.

10. Wang X, Tomso DJ, Liu X and Bell DA: Single nucleotide polymorphism in transcriptional regulatory regions and expression of environmentally responsive genes. Toxicol App Pharmacol 207: 84-90, 2005.

11. Jadidi N, Alesaeidi S, Arab F, Pakzad B, Siasi E and Esmaeilzadeh E: MiRNA-binding site polymorphism in IL-15RA gene in rheumatoid arthritis and systemic lupus erythematosus: Correlation with disease risk and clinical characteristics. Clin Rheumatol 41: 3487-3494, 2022.

12. Zeinalzadeh S, Kheradmand N, Rasouli G, Esmaeilzadeh E, Pakzad B, Behroozi J, Chamanara M, Zoshk MY, Ehtesham N and Sabet MN: Association of a miRNA-binding site polymorphism in IL-16 gene with disease risk and clinical characteristics of rheumatoid arthritis and systemic lupus erythematosus. Clin Rheumatol 41: 2189-2196, 2022.

13. Chen X, Ding X, Wu Q, Qi J, Zhu M and Miao C: Monomethyltransferase SET8 facilitates hepatocellular carcinoma growth by enhancing aerobic glycolysis. Cell Death Dis 10: 312, 2019.

14. Fang J, Feng Q, Ketel CS, Wang H, Cao R, Xia L, Erdjument-Bromage H, Tempst P, Simon JA and Zhang Y: Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. Curr Biol 12: 1086-1099, 2002.

15. Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang Y, Chuikov S, Valenzuela P, Tempst P, Steward R, *et al*: PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Mol Cell 9: 1201-1213, 2002.

16. Wu S, Wang W, Kong X, Congdon LM, Yokomori K, Kirschner MW and Rice JC: Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes Dev 24: 2531-2542, 2010.

17. Singh V, Prakhar P, Rajmani RS, Mahadik K, Borbora SM and Balaji KN: Histone methyltransferase SET8 epigenetically reprograms host immune responses to assist mycobacterial survival. J Infect Dis 216: 477-488, 2017.

18. Wang XP, Cheng QY, Gu MM, Leng RX, Fan YG, Li BZ and Ye DQ: Diagnostic accuracy of anti-keratin antibody for rheumatoid arthritis: A meta-analysis. Clin Rheumatol 38: 1841-1849, 2019.

19. Moll R, Divo M and Langbein L: The human keratins: Biology and pathology. Histochem Cell Biol 129: 705-733, 2008.

20. Sha Z, Lai R, Zhang X, Zhao Y, Wu J, Geng C and Guo Z: A polymorphism at the microRNA binding Site in the 3' untranslated region of KRT81 is associated with breast cancer. DNA and Cell Biol 39: 1886-1894, 2020.

21. Zhang K, Liang Y, Zhang W, Zeng N, Tang S and Tian R: KRT81 knockdown inhibits malignant progression of melanoma through regulating interleukin-8. DNA Cell Biol 40: 1290-1297, 2021.

22. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, *et al*: 2010 Rheumatoid arthritis classification criteria: An American college of rheumatology/European league against rheumatism collaborative initiative. Arthritis Rheum 62: 2569-2581, 2010.

23. Fransen J and van Riel PL: The disease activity score and the EULAR response criteria. Rheum Dis Clin North Am 35: 745-757, vii-viii, 2009.

24. Mukhopadhyay K, De S, Kundu S, Ghosh P, Chatterjee S and Chatterjee M: Evaluation of levels of oxidative stress as a potential biomarker in patients with rheumatoid arthritis. J Family Med Prim Care 10: 1981-1986, 2021.

25. Mateen S, Moin S, Khan AQ, Zafar A and Fatima N: Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS One 11: e0152925, 2016.

26. He L and Hannon GJ: MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 5: 522-531, 2004.

27. Esteller M: Non-coding RNAs in human disease. Nat Rev Gene 12: 861-874, 2011.

28. Zhou M, Jiang B, Xiong M and Zhu X: An updated meta-analysis of the associations between microRNA polymorphisms and susceptibility to rheumatoid arthritis. Front Physiol 9: 1604, 2018.

29. Papathanasiou I, Mourmoura E, Balis C and Tsezou A: Impact of miR-SNP rs2910164 on miR-146a expression in osteoarthritic chondrocytes. Adv Med Sci 65: 78-85, 2020.

30. Song F, Zheng H, Liu B, Wei S, Dai H, Zhang L, Calin GA, Hao X, Wei Q, Zhang W and Chen K: An miR-502-binding site single-nucleotide polymorphism in the 3'-untranslated region of the SET8 gene is associated with early age of breast cancer onset. Clin Cancer Res 15: 6292-6300, 2009.

31. Chen X, Wu Q, Jiang H, Wang J, Zhao Y, Xu Y and Zhu M: SET8 is involved in the regulation of hyperglycemic memory in human umbilical endothelial cells. Acta Biochim Biophys Sin(Shanghai) 50: 635-642, 2018.

32. Balogh E, Veale DJ, McGarry T, Orr C, Szekanecz Z, Ng CT, Fearon U and Biniecka M: Oxidative stress impairs energy metabolism in primary cells and synovial tissue of patients with rheumatoid arthritis. Arth Res Ther 20: 95, 2018.

33. Mateen S, Moin S, Zafar A and Khan AQ: Redox signaling in rheumatoid arthritis and the preventive role of polyphenols. Clin Chim Acta 463: 4-10, 2016.

34. Bauerová K and Bezek A: Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. Gen Physiol Biophys 18: 15-20, 1999.

35. Broeren MG, de Vries M, Bennink MB, Arntz OJ, Blom AB, Koenders MI, van Lent PL, van der Kraan PM, van den Berg WB and van de Loo FA: Disease-regulated gene therapy with anti-inflammatory interleukin-10 under the control of the CXCL10 promoter for the treatment of rheumatoid arthritis. Hum Gene Ther 27: 244-254, 2016.

36. Tian S, Yan Y, Qi X, Li X and Li Z: Treatment of type II collagen-induced rat rheumatoid arthritis model by interleukin 10 (IL10)-mesenchymal stem cells (BMSCs). Med Sci Monit 25: 2923-2934, 2019.

37. Henningsson L, Eneljung T, Jirholt P, Tengvall S, Lidberg U, van den Berg WB, van de Loo FA and Gjertsson I: Disease-dependent local IL-10 production ameliorates collagen induced arthritis in mice. PLoS One 7: e49731, 2012.