Journal of Inflammation Research

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Uncovering Circular RNA Signatures in Rheumatoid Arthritis: Novel Biomarkers and Clinical Implications

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Background: Although increasing evidence implicates circular RNAs (circRNAs) in the pathogenesis of various autoimmune diseases, the potential role of circRNAs in rheumatoid arthritis (RA) remains poorly understood.

Methods: Utilizing a two-phase study design, we performed high-throughput RNA sequencing on peripheral blood mononuclear cells (PBMCs) from three treatment-naïve RA patients and three healthy controls (HCs), followed by validation in an independent cohort of 32 RA patients and 32 HCs using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: A total of 157 circRNAs were differentially expressed between RA patients and HCs, including 91 upregulated and 66 downregulated circRNAs. Six circRNAs, including hsa_circ_0001394, hsa_circ_0001998, hsa_circ_0009172, hsa_circ_0006732, circRNA2842, and circRNA8330, were further confirmed to be upregulated in RA. Among them, hsa_circ_0001394 demonstrated perfect sensitivity (100%), and hsa_circ_0001998 exhibited perfect specificity (100%) in distinguishing RA from HCs. All six circRNAs showed considerable diagnostic performance, with area under the curve (AUC) values ranging from 0.794 to 0.993. Additionally, specific circRNAs correlated significantly with established serological markers: hsa_circ_0009172 expression was negatively associated with anti-cyclic citrullinated peptide (anti-CCP) antibody titers, whereas hsa_circ_0001998 expression correlated positively with rheumatoid factor (RF). Pathway enrichment analysis suggested that these differentially expressed circRNAs may participate in RA pathogenesis through key pathways like Wnt, calcium and VEGF signaling.

Conclusion: Our study identifies several novel circRNAs with high diagnostic utility for RA, highlighting their potential as promising biomarkers and therapeutic targets.

Keywords: rheumatoid arthritis, circular RNAs, PBMCs, biomarker

Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial inflammation, progressive joint destruction, and systemic multi-organ involvement.[1] The pathogenesis of RA involves a complicated interaction of environmental, epigenetic, and genetic factors. Genetic susceptibility is largely attributed to both human leukocyte antigen (*HLA*) (eg, *DRB1*04*) and non-*HLA* genes (eg, *PTPN22, STAT4*). Given that RA is a polygenic disorder with an estimated heritability of approximately 60%, the epigenetic mechanisms, such as DNA methylation and microRNAs (miRNAs) regulation, are thought to account for part of its "missing heritability".[2] Environmental exposures, including smoking and psychological stress, could induce post-translational protein modifications and immune cell activation, stimulating lymphoid aggregation in synovium and amplifying local and systemic inflammation.[3] Despite extensive research, no single factor could fully explain the diverse clinical features of RA. Current studies mainly focus on aberrant immune cell activation, dysregulated cytokine networks, and joint tissue repair mechanisms. However, major clinical challenges persist, including marked heterogeneity in disease

Received: 20 June 2025
Accepted: 23 November 2025
Published: 16 December 2025

course, difficulties in early diagnosis, and variations in individual treatment responses to targeted therapies.[4–6] Conventional serological biomarkers, such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibody, are clinically valuable; however, their sensitivity and diagnostic consistency are limited, particularly in early or seronegative RA. Moreover, they do not reliably capture dynamic disease activity or treatment response.[7] Therefore, identification of early diagnostic biomarkers with high sensitivity and specificity is crucial for improving disease diagnosis, treatment selection, and outcome evaluation.[8]

Circular RNAs (circRNAs) are a unique class of covalent closed non-coding RNAs (ncRNAs) characterized by high stability, resistance to exonuclease degradation in body fluid, and tissue- or developmental stage-specific expression.[9] Functionally, circRNAs modulate inflammatory responses, immune activation, and synovial hyperplasia through competing endogenous RNA (ceRNA) mechanisms, transcriptional regulation, or interactions with RNA-binding proteins.[10] Given these properties, circRNAs have gained increasing attention for their therapeutic potential. Accumulating evidence demonstrates that dysregulated circRNA expression may contribute to the pathogenesis of several autoimmune diseases, including RA, systemic lupus erythematosus (SLE), and primary biliary cholangitis (PBC).[11–13]

Recent advances in high-throughput RNA sequencing and microarray technologies have revealed aberrant circRNA expression profiles in various tissues and cell types derived from RA patients, such as plasma, peripheral blood mononuclear cells (PBMCs), fibroblast-like synoviocytes (FLS), and synovial tissues.[14] For instance, comparative analyses identified 35,342 differentially expressed circRNAs (DEcircRNAs) in RA patients than healthy controls (HCs).[15] Similarly, microarray profiling of RA PBMCs revealed 149 upregulated and 250 downregulated circRNAs relative to HCs.[16] Functionally, circ_0083964 was found to exacerbate synovial hyperplasia and inflammation via the circ_0083964/miR-204-5p/YY1 axis.[17] While circ_0004712 enhanced FLS invasiveness by sponging miR-633 and activating the TRAF6/NF-κB pathway.[18] Conversely, circ_0008360, downregulated in RA synovial tissues, modulates RA-FLS migration, apoptosis, proliferation, and inflammatory responses via sponging miR-135b-5p and reducing histone deacetylase 4 (HDAC4).[19] Despite these findings, circRNAs expression patterns and pathogenic roles in RA remain controversial with poorly understand, necessitating further investigation into their mechanistic contributions to RA pathogenesis.

PBMCs, consisting mainly of lymphocytes and monocytes, are central mediators of immune responses and play a pivotal role in RA pathogenesis. The aberrant activation of PBMCs and the caused cytokine production dysregulation contribute to chronic inflammation and joint destruction. Furthermore, PBMCs can be easily obtained via venipuncture, offering a minimally invasive yet effective source for exploring immune alterations.[20] Based on these considerations, we employed high-throughput RNA sequencing technology combined with real-time quantitative polymerase chain reaction (RT-qPCR) validation to systematically identify and validate DEcircRNAs in PBMCs of RA patients, to provide novel molecular candidates for early diagnosis and potential therapeutic targeting of RA.

Materials and Methods

Participants and Data Collection

All participants were enrolled from the Department of Rheumatology and Immunology, the First Affiliated Hospital of Anhui Medical University, between October 2022 and January 2023. In the discovery phase, peripheral blood samples were obtained from three newly diagnosed, treatment naïve RA patients and three age-sex-matched HCs for high-throughput RNA sequencing. In the subsequent validation phase, we included 32 RA patients and 32 age-sex-matched HCs. The diagnosis of RA was established according to the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) classification criteria 2010.[21] Participants with pregnancy, concurrent infections, other autoimmune or systemic diseases, or any history of malignancy were excluded. Demographic and clinical information was obtained from medical records and standardized health questionnaires.

All participants provided written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

RNA Extraction

Peripheral blood (10 mL) was collected from each donor. PBMCs were isolated using Ficoll density gradient centrifugation and stored at −80°C until use. Total RNA was extracted from PBMCs using Trizol reagent (Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. The purity and concentration of RNA were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, MA, USA).

High-Throughput RNA Sequencing

Comprehensive transcriptome RNA sequencing was performed by LC-Bio Technology Co., Ltd. (Hangzhou, China) using the Illumina HiSeq 2500 platform. DEcircRNAs were identified using a threshold $P < 0.05$, and $\log_2$ fold change (FC)> 1.0. Cluster analysis was conducted to visualize expression pattern differences across the tested specimens.

DEcircRNAs Validation by RT-qPCR

Complementary DNA (cDNA) was synthesized from total RNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). After that, we performed RT-qPCR using Novostart SYBR qPCR SuperMix Plus (Novoprotein, Suzhou, China). Relative expression levels were computed utilizing the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Continuous variables with normal distribution were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables were presented as median (interquartile range, IQR). Categorical variables were summarized as number and percentages. Differences between two groups were assessed using the independent-sample *t*-test for normally distributed variables or the Mann–Whitney *U*-test for non-normally distributed variables. Receiver operating characteristic (ROC) curve analysis was applied to evaluate the diagnostic performance of circRNAs, and the area under the curve (AUC) was calculated. Correlations between circRNA expression and clinical parameters were analyzed using Spearman's rank correlation analysis. Linear regression analysis was further performed to assess the association between RA serological markers and candidate circRNAs. A $P < 0.05$ was considered statistically significant.

All statistical analyses and data visualization were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA), respectively.

Results

Characteristics of Study Subjects

A total of 35 RA patients and 35 HCs were enrolled. Among them, three newly diagnosed, treatment naïve RA patients and three HCs were recruited in the discovery phase, and the remaining 32 RA patients and 32 HCs were included in the validation phase. The demographic and clinical characteristics of the participants are summarized in Table 1.

Table 1 Demographic and Clinical Characteristics of RA Patients and Healthy Controls

Phase	Demographic and Clinical Characteristics	RA	HC	*P*
Screening	n	3	3	
	Age (years, mean±SD)	43.35±8.88	45.67±13.65	0.817
	Gender (male/female)	0/3	0/3	1.000
	Disease duration (years, mean±SD)	3.49±2.42	/	
	ESR (mm/h, mean±SD)	50.00±36.06	/	
	CRP (mg/L, mean±SD)	50.98±54.48	/	
	RF (IU/mL, mean±SD)	91.23±89.79	/	
	Anti-CCP (U/mL, mean±SD)	300.33±492.71	/	
	DAS28 (mean±SD)	4.41±0.68	/	

(*Continued*)

Table 1 (Continued).

Phase	Demographic and Clinical Characteristics	RA	HC	*P*
Validation	n	32	32	
	Age [years, M (P25, P75)]	58.34 (48.57,61.20)	51.00(44.25,62.75)	0.361
	Gender (male/female)	9/32	6/32	0.380
	Disease duration [years, M (P25, P75)]	5.62(1.05,11.78)	/	
	ESR [mm/h, M (P25, P75)]	38.00(22.25,47.50)	/	
	CRP [mg/L, M (P25, P75)]	18.05(5.21,32.82)	/	
	RF [IU/mL, M (P25, P75)]	78.85(40.25,125.28)	/	
	Anti-CCP [U/mL, M (P25, P75)]	313.50(102.75, 1299.75)	/	
	DAS28 (mean±SD)	4.16±1.33	/	

Abbreviations: Anti-CCP, anticyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity score of 28 joints; ESR, erythrocyte sedimentation rate; HC, healthy control; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation.

CircRNAs Expression Profiles

Comprehensive transcriptome sequencing identified 91 upregulated and 66 downregulated circRNAs in RA patients compared with HCs (Figure 1). Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed that the DEcircRNAs were significantly enriched in the vascular endothelial growth factor (VEGF), Wnt and calcium signaling pathways. Gene Ontology analysis demonstrated that the biological process of these DEcircRNAs are mainly involved in transcriptional regulation (DNA template), signal transduction, protein modification (phosphorylation/ubiquitination), and apoptosis regulation. The cell components were primarily located in the nucleus, membrane system (plasma membrane/endoplasmic reticulum), and intracellular vesicles (exosomes). The molecular functions include protein/nucleic acid binding and kinase/transferase activity (Figure 2).

Validation of DEcircRNAs by qRT-PCR

Based on the threshold $P < 0.05$ and $|\log_2 FC| > 1$, six DEcircRNAs (hsa_circ_0001394, hsa_circ_0001998, hsa_circ_0009172, hsa_circ_0006732, circRNA2842, and circRNA8330) were selected for validation. Total RNA was extracted from PBMCs of 32 RA patients and 32 HCs, and specific primers were designed for each target. The results showed significantly higher expression levels of all six DEcircRNAs in RA patients compared with HCs (Table 2) (Figure 3).

Diagnostic Value of Six DEcircRNAs

ROC curve analysis was performed to evaluate the diagnostic potential of the six DEcircRNAs. As shown in Table 3 and Figure 4, the AUC values ranged from 0.7944 (95% CI: 0.6880, 0.9009) for circRNA2842 to 0.9932 (95% CI: 0.9809, 1.000) for hsa_circ_0001998. Sensitivity ranged from 81.25% (circRNA2842 and circRNA 8330) to 100.00% (has_circ_0001394), and specificity varied from 62.5% (circRNA2842) to 100.00% (hsa_circ_0001998).

Associations Between the Six DEcircRNAs and Clinical Indicators of RA

Correlation analysis between six DEcircRNAs and clinical indicators in RA patients is presented in Table 4. The expression level of hsa_circ_0001998 showed a positive correlation with RF ($rs = 0.414$, $P = 0.018$), while hsa_circ_0009172 expression level was negatively associated with anti-CCP antibody titers ($rs = -0.373$, $P = 0.036$). Linear regression analysis further revealed that hsa_circ_0009172 was independently associated with anti-CCP antibody titers ($\beta = -417.225$, $P = 0.043$) (Table 5).

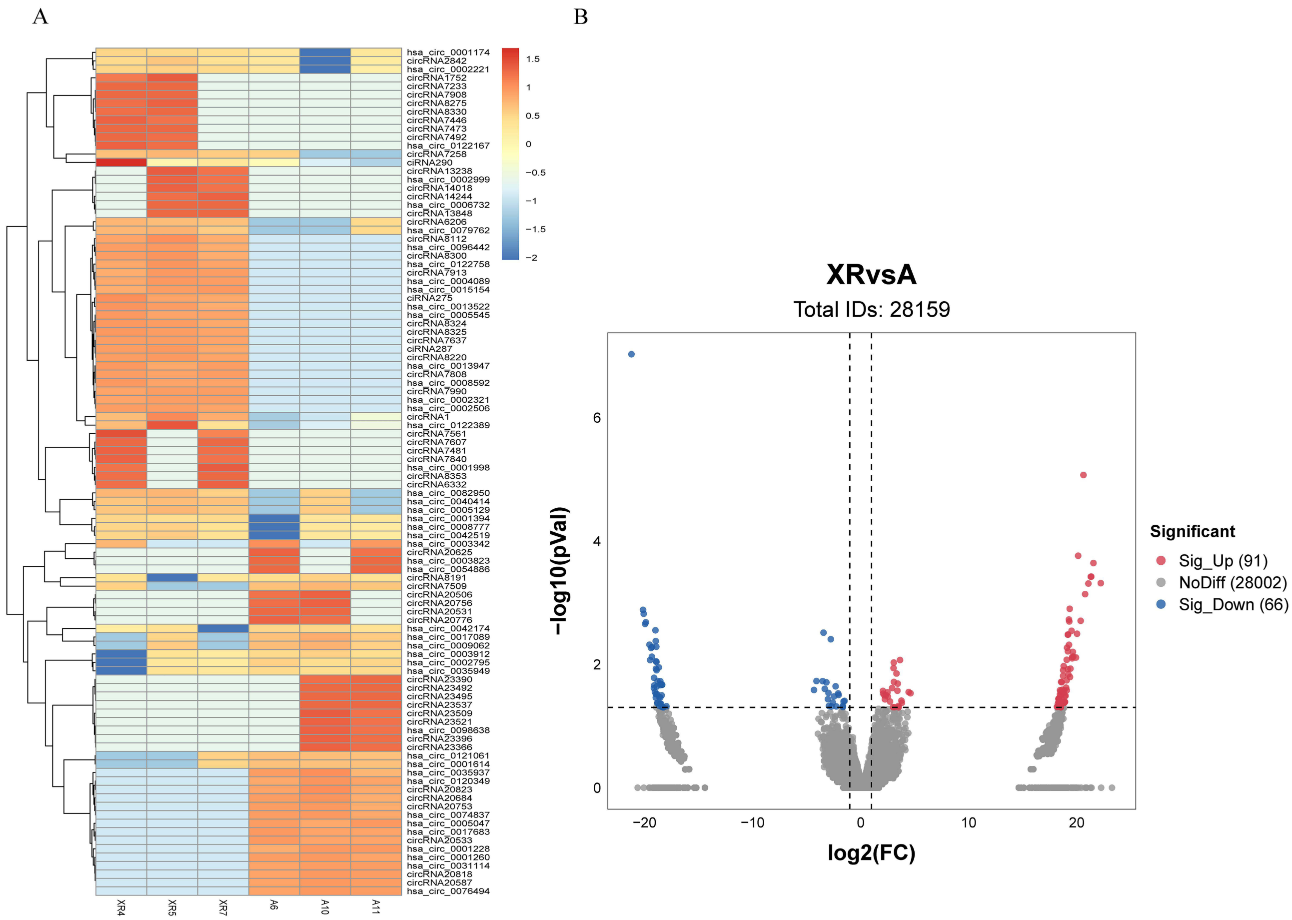

Figure 1 Identification of differentially expressed circRNAs between RA patients and HCs. (**A**) DEcircRNAs heat map. Red rectangles indicate high expression, blue rectangles indicate low expression, and grey indicates non-significant; (**B**) DEcircRNAs volcano plot. Red plots indicate high expression, blue plots indicate low expression, and grey plots indicate non-significant.

Abbreviations: circRNAs, circular RNAs; RA, rheumatoid arthritis; HC, healthy controls; DE, differentially expressed.

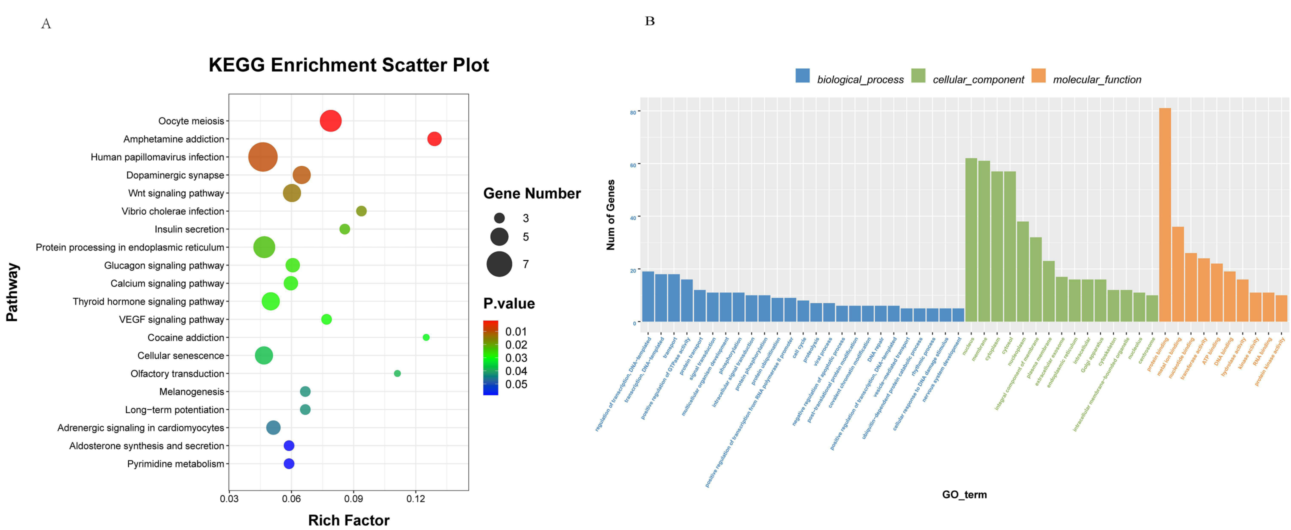

Figure 2 Enrichment analysis of the genes associated with circRNAs. (**A**) KEGG pathway analysis; (**B**) GO enrichment analysis terms (biological processes, cellular components, molecular function).

Abbreviations: circRNAs, circular RNAs; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Table 2 The Expression of 6 Candidate circRNAs Between RA (n = 32) and HCs (n = 32) in the Validation Phase

circRNA	RA	HCs	*P*
has_circ_0001394	2.81(2.33, 3.80)	1.18(0.66, 1.50)	<0.001*
hsa_circ_0001998	3.69(2.98, 5.47)	1.07(0.66, 1.50)	<0.001*
hsa_circ_0009172	3.54(2.53, 4.31)	1.08(0.54, 1.89)	<0.001*
hsa_circ_0006732	2.12(1.60, 2.52)	1.18(0.63, 1.56)	<0.001*
circRNA2842	1.82(1.28, 3.81)	1.12(0.62, 1.52)	<0.001*
circRNA8330	2.11(1.72, 3.00)	1.03(0.78, 1.40)	<0.001*

Notes: *: the result of the Mann–Whitney *U*-test between RA and HC group.
Abbreviations: HC, healthy control; RA, rheumatoid arthritis.

Discussion

Although RF and anti-CCP antibody in RA remain valuable diagnostic biomarkers for RA, their diagnostic utility is not absolute. While anti-CCP antibody exhibits high specificity, the sensitivity of both RF and anti-CCP varies considerably, particularly in early or seronegative disease.[7] This limitation hinders efforts toward accurate molecular classification and precision medicine. Therefore, identifying novel biomarkers with high sensitivity and specificity is critical for improving early diagnosis and individualized treatment.

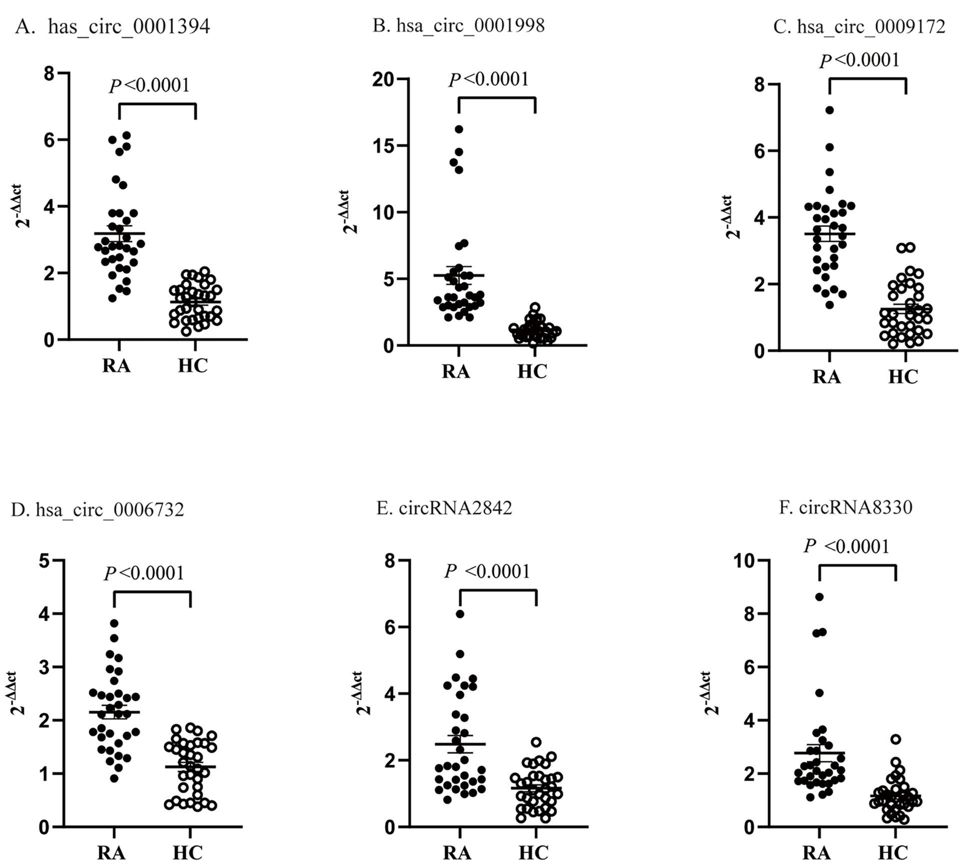

Figure 3 The expression levels of upregulated circRNAs between RA and HCs. (**A**) hsa_circ_0001394; (**B**) hsa_circ_0001998; (**C**) hsa_circ_0009172; (**D**) hsa_circ_0006732; (**E**) circRNA2842; and (**F**) circRNA8330.
Abbreviations: circRNAs, circular RNAs; RA, rheumatoid arthritis; HC, healthy controls.

Table 3 Potential Diagnostic Value of the Six DEcircRNAs for RA

circRNA	AUC	95% CI	*P*	Sensitivity (%)	Specificity (%)
has_circ_0001394	0.9604	(0.9182,1.000)	<0.0001	100	84.38
hsa_circ_0001998	0.9932	(0.9809,1.000)	<0.0001	93.75	100
hsa_circ_0009172	0.9380	(0.8850,0.9910)	<0.0001	93.75	81.25
hsa_circ_0006732	0.8730	(0.7898,0.9563)	<0.0001	87.5	75
circRNA 2842	0.7944	(0.6880,0.9009)	<0.0001	81.25	62.5
circRNA 8330	0.8901	(0.8091,0.9712)	<0.0001	81.25	90.63

Abbreviations: AUC, area under the curve; CI, confidence interval; RA, rheumatoid arthritis; ROC, receiver operating characteristic curve.

Emerging evidence highlights circRNAs as a crucial class of regulatory ncRNAs that modulate gene expression through multiple mechanisms, such as acting as ceRNAs, regulating transcription, and interacting with RNA-binding proteins.[22–25] Recent studies have demonstrated cirRNAs are involved in the pathogenesis of RA.[26] In the present study, we identified a distinct circRNAs expression profile in PBMCs of RA patients, comprising 91 upregulated and 66 downregulated circRNAs compared with HCs. Six up-regulated cirRNAs (hsa_circ_0001394, hsa_circ_0001998,

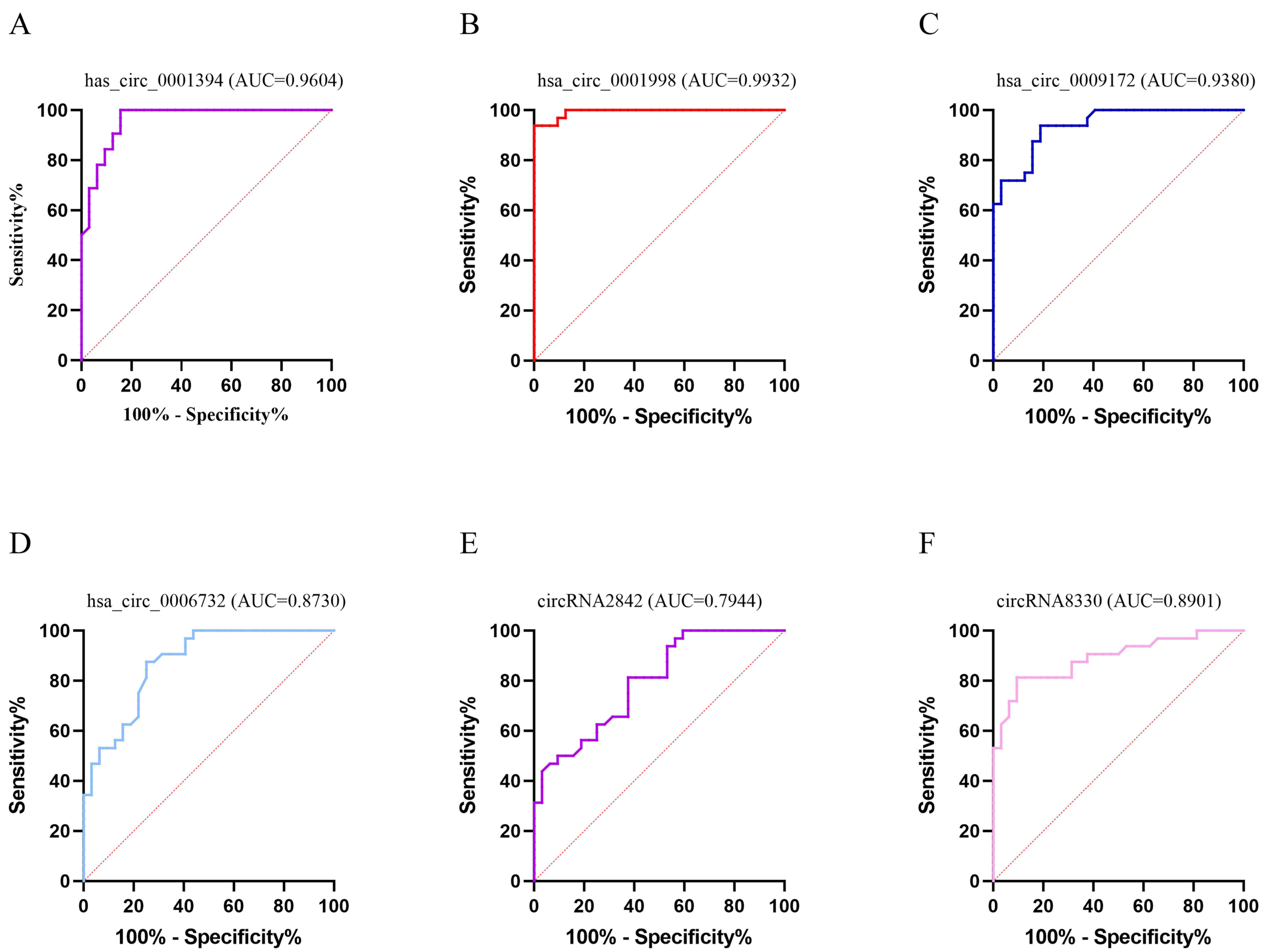

Figure 4 Diagnostic values of the six DEcircRNAs. (**A**) hsa_circ_0001394; (**B**) hsa_circ_0001998; (**C**) hsa_circ_0009172; (**D**) hsa_circ_0006732; (**E**) circRNA2842; and (**F**) circRNA8330.

Abbreviations: circRNAs, circular RNAs; RA, rheumatoid arthritis; HC, healthy controls; DE, differentially expressed.

https://doi.org/10.2147/JIR.S548400

Table 4 Relationship Between the Six DEcircRNAs and RA-Related Clinical Indices

Clinical Index	Has_circ_0001394		Hsa_circ_0001998		Hsa_circ_0009172		Hsa_circ_0006732		circRNA2842		circRNA8330	
	rs	*P*	*rs*	*P*	*rs*	*P*	*rs*	*P*	*rs*	*P*	*rs*	*P*
ESR	0.098	0.595	0.116	0.526	−0.015	0.937	0.241	0.184	0.073	0.690	0.138	0.451
CRP	−0.010	0.955	0.062	0.735	0.082	0.657	−0.140	0.444	0.047	0.800	0.217	0.233
RF	−0.009	0.960	0.414	0.018*	0.087	0.636	0.091	0.621	0.214	0.239	0.215	0.236
Anti-CCP	−0.054	0.770	−0.176	0.336	−0.373	0.036*	−0.154	0.399	−0.018	0.922	0.190	0.298
DAS28	0.163	0.372	0.021	0.911	0.176	0.335	0.285	0.114	−0.120	0.512	−0.190	0.297

Note: *: $P < 0.05$.
Abbreviations: Anti-CCP, antibody against cyclic citrulline polypeptide; CRP, C reactive protein; DAS28, disease activity score of 28 joints; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

Table 5 Multiple Linear Regression Analysis of RF and Anti-CCP

Dependent Variable	Candidate circRNA	Nonnormalized *B*	Coefficient SE	Standardization Coefffcient *B*	t	*P*	VIF
RF	hsa_circ_0001998	14.477	8.178	0.308	1.770	0.087	1.000
Anti-CCP	hsa_circ_0009172	−417.225	197.822	−0.359	−2.109	0.043*	1.000

Note: *: $P < 0.05$.
Abbreviations: Anti-CCP, antibody against cyclic citrulline polypeptide; B, regression slope coefficient; RF, rheumatoid factor; SE, standard error; VIF, variance inflation factor.

hsa_circ_0009172, hsa_circ_0006732, circRNA2842, and circRNA8330) were further validated in an independent cohort of 32 RA patients and 32 HCs. Notably, these circRNAs demonstrated promising diagnostic potential: hsa_circ_0001394 exhibited the highest sensitivity, whereas hsa_circ_0001998 demonstrated the superior specificity. Furthermore, hsa_circ_0001998 and hsa_circ_0009172 were significantly correlated with several RA clinical indicators, suggesting that circRNAs may serve as novel biomarkers for the diagnosis of RA.

KEGG enrichment analysis indicated that the DEcircRNAs were enriched in several key signaling pathways, particularly the Wnt pathway, calcium pathway and VEGF pathway, which are all critically implicated in RA pathogenesis. Dysregulation of the Wnt/β-catenin signaling disrupts the osteoblast-osteoclast balance and promotes bone erosion. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), induce the expression of bone formation inhibitors Dickkopf-1 (Dkk-1) and sclerostin, thereby suppressing bone formation and enhancing resorption.[27] The non-canonical Wnt/planar cell polarity (PCP) pathway is also activated in RA and contributes to synovial proliferation and bone destruction.[28] Moreover, non-canonical Wnt signaling intersects with calcium-dependent pathways by activating Ca^2+/calcineurin/Nuclear factor of activated T-cells (NFAT) cascades. The Ca^2+-calcineurin-NFAT signaling axis exhibits dual pathogenic roles in RA pathogenesis. First, it drives aberrant immune activation via enhanced Th17 differentiation and synovial fibroblast aggression, thereby causing excessive production of pro-inflammatory cytokines, such as interleukin (IL)-17, and matrix metalloproteinases (MMPs). Second, it perpetuates chronic joint inflammation and structural damage through these effector mechanisms. The therapeutic efficacy of calcineurin inhibitors (eg, cyclosporin A) in RA, which modulate both immune and stromal compartments, underscores the central role of calcium-dependent signaling in RA.[29] Interestingly, cyclosporin A exhibits dose-dependent effects on bone metabolism: low doses promote osteogenesis through Fos-related antigen (Fra)-2-mediated transcriptional activation, whereas high doses inhibit osteogenic differentiation.[30] In addition, the calcineurin-NFAT pathway regulates neutrophil antimicrobial activity; its inhibition impairs bactericidal capacity and promotes immunosuppressive phenotypes, which may explain the increased risk of coinfection in RA.[31] Concurrently, VEGF-driven angiogenesis in RA synovium amplifies inflammation and joint destruction, consistent with previous studies showing that VEGF inhibition (eg, via β-sitosterol) attenuates disease severity in preclinical models.[32,33]

Among the DEcircRNAs, three (hsa_circ_0001394, hsa_circ_0001998, and hsa_circ_0006732) have been previously linked to cancer, but their potential involvement in RA pathogenesis was first revealed in this study. In contrast, hsa_circ_0009172, circRNA2842, and circRNA8330 have not been reported in any disease.

Hsa_circ_0001394, a 740 base pair (bp) circular RNA derived from TBC1 domain family member 14 (*TBC1D14*) gene locus, exhibits exceptional stability due to its covalently closed structure, rendering it resistant to RNase R degradation. Elevated hsa_circ_0001394 expression has been reported in postmenopausal osteoporosis and hepatocellular carcinoma (HCC).[34,] Mechanistically, hsa_circ_0001394 acts as a ceRNA for miR-527 to derepress ubiquitin-conjugating enzyme E2A (UBE2A) expression, thereby promoting HCC.[35] Given that TBC1D14 regulates autophagosome formation via Ras-related protein Rab-11 (Rab11) and Unc-51-like kinase 1 (ULK1),[36] this finding is particularly relevant, as autophagy plays dual roles in RA, maintaining cellular homeostasis under normal conditions but promoting synovial hyperplasia and inflammation when excessively activated.[37]

Similarly, previous studies have demonstrated the upregulated expression of hsa_circ_0001998 in colorectal carcinoma tissues[38] and a functional part in promoting pulmonary adenocarcinoma progression through the miR-145 axis.[39] In our study, we observed elevated expression of hsa_circ_0001998 in RA PBMCs, suggesting a potential role in amplifying inflammatory signaling pathways in RA.

Hsa_circ_0006732 is significantly enhanced in colorectal carcinoma and promotes tumor progression by acting as a ceRNA for miR-127-3p and regulating Ras-related protein Rab-3D (RAB3D).[40] This observation is particularly interesting considering rat sarcoma virus(Ras)-related signaling pathways, including the RhoA/Rho-Kinase (ROCK) axis, participate in synovial fibroblast activation and joint destruction in RA.[41] Upregulation of hsa_circ_0006732 in RA PBMCs suggests a potential role in similar pathways, which regulate synovial hyperplasia and invasive behaviors.

Hsa_circ_0009172 is a circular RNA derived from the DNA replication helicase/nuclease 2 (*DNA2*) gene. *DNA2*, a gene encoding a helicase/nuclease involved in DNA repair and replication, has been implicated in various neoplastic diseases due to its deficiency or mutations.[42] In RA, the CG site of *DNA2* (chr10:70231628) was identified as one of 16 critical methylation markers, with its methylation level significantly correlating with clinical indicators, suggesting a potential role in RA pathogenesis.[43] Future research should determine whether hsa_circ_0009172 influences immune dysregulation through modulation of its parental gene or independent ceRNA activity.

The abnormal migration and invasion of FLS are the key factors causing cartilage destruction in RA. This invasive behavior relies on the extracellular signal integration and the remodeling of the actin cytoskeleton. CircRNA2842 is a circular RNA molecule generated by splicing the proline-serine-threonine phosphatase interacting protein 2 (*PSTPIP2*) gene. PSTPIP2 expression level was negatively associated with osteoclast development, suggesting it might moderate disease. In autoinflammatory disease models, the absence of *PSTPIP2* can lead to phenotypes similar to Synovitis-Acne-Pustulosis-Hyperostosis-Osteitis (SAPHO) syndrome; in RA, PSTPIP2 can regulate the dynamic changes of synovial macrophages through estrogen receptor β (ERβ).[44] Macrophages with high expression of PSTPIP2 (F4/80+PSTPIPhi) are prone to form pseudopodia and migrate to the inner layer of the synovial membrane, constituting a protective immune barrier that inhibits bone erosion.[45] Future studies need to further verify whether circRN2842 is directly involved in the aforementioned process and unveil its interaction with known pathways, such as the Janus kinase-signal transducer and activator of transcription JAK/STAT or VEGF signaling pathways.

CircRNA8330, generated from the NLR family pyrin domain containing 12 (*NLRP12*) gene, displayed marked upregulation in RA patients. Studies have shown that the protein product encoded by circRNA8330 acts as a dual modulating effect in innate immune response: on the one hand, it inhibits anti-inflammatory effects via restraining NF-kappaB (NF-κB) signaling pathway; on the other hand, it acts as a molecular switch for inflammasome activation to participate in pro-inflammatory responses. Loss-of-function mutations of *NLRP12* gene (such as T284X, R352C) can abolish its inhibitory effects on NF-κB signaling pathway, thereby causing excessive activation of this signaling cascade, contributing to autoinflammatory syndromes such as NLRP12-associated autoinflammatory disease (NLRP12-AID).[46] Furthermore, NLRP12 frameshift mutations can significantly promote generation of pro-inflammatory factors via abnormally activating ERK and NF-κB signaling pathways in pathological process of intestinal inflammation in Crohn's disease.[47] In SLE, it was observed that NLRP12 expression level in PBMCs of patients was lower than HCs group. Correlation analysis indicated that low NLRP12 expression was negatively associated with disease activity and the deterioration of renal function. Further mechanism studies have revealed that an inverse feedback moderating loop formed between NLRP12 protein and type I interferon (IFN-I) may have exacerbated the pathological process of lupus nephritis (LN).[48] In RA, NLRP12 effectively inhibits the abnormal proliferation and inflammatory factor secretion of RA-FLS by synergically regulating NF-κB and MAPK signaling pathways.[49] These findings indicate that NLRP12 and its derivative circRNA8330 play crucial immunomodulatory roles in autoimmune diseases.

Despite the valuable insights, several limitations should be acknowledged. First, the relatively small sample size, single-center recruitment, and potential regional bias may limit the generalizability of our findings and reduce statistical power. Second, the lack of functional validation of candidate circRNAs restricts our understanding of their precise biological effects. Future studies should employ in vitro and in vivo models to elucidate their molecular mechanisms. Third, the diagnostic potential of the observed circRNAs should be further validated in larger, multi-center, and multi-ethnic cohorts. Future longitudinal studies are warranted to assess the values of these circRNAs on disease progression, therapeutic response, or remission status over time.

Conclusions

This study established a circRNAs expression profile in RA PBMCs and identified six circRNAs (hsa_circ_0001394, hsa_circ_0001998, hsa_circ_0009172, hsa_circ_0006732, circRNA2842, and circRNA8330) that were associated with key clinical indicators of RA and demonstrated considerable diagnostic values, suggesting the potential of circRNAs as non-invasive biomarkers for RA. Further functional validation and studies with larger, multi-center cohorts are needed to confirm their clinical utility and explore their mechanisms in RA pathogenesis.

Abbreviations

Anti-CCP, anti-cyclic citrullinated peptide; AUC, area under the curve; bp, base pair; circRNAs, circular RNAs; ceRNA, competing endogenous RNA; DEcircRNAs, differentially expressed circRNAs; Dkk-1, Dickkopf-1; DNA2, DNA replication helicase/nuclease 2; Erβ, estrogen receptor β; Fra-2, Fos-related antigen 2; FLS, fibroblast-like synoviocytes; HCs, healthy controls; HCC, hepatocellular carcinoma; HDAC4, histone deacetylase 4; IFN-I, type I interferon; IL, interleukin; JAK/STAT, Janus kinase-signal transducer and activator of transcription; LN, lupus nephritis; miRNAs, microRNAs; MMPs, matrix metalloproteinases; ncRNAs, non-coding RNAs; NLRP12, NLR family pyrin domain containing 12; NFAT, Nuclear factor of activated T-cells; NF-κB, NF-kappaB; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; PCP, planar cell polarity; PSTPIP2, proline-serine-threonine phosphatase interacting protein 2; RA, rheumatoid arthritis; Rab11, Rab-11; Ras, rat sarcoma virus; RAB3D, Ras-related protein Rab-3D;RF, rheumatoid factor; ROCK, Rho-Kinase;RT-qPCR, real-time quantitative polymerase chain reaction; SLE, systemic lupus erythematosus; TNF-α, tumor necrosis factor alpha; ubiquitin-conjugating enzyme E2A (UBE2A); VEGF, vascular endothelial growth factor; ULK1, Unc-51-like kinase 1.

Data Sharing Statement

Available on request from the corresponding author.

Ethical Statement

This study was conducted in compliance with the ethical guidelines of the Ethics Committee of The First Affiliated Hospital of Anhui Medical University (approval no. 2023775) and adhered to the principles of the 1964 Helsinki Declaration and its subsequent amendments.

Consent for Publication

Informed consent of all participants was obtained.

Acknowledgments

We would like to thank all the patients with RA who participated in this study.

Author Contributions

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

Funding

This research was funded by Basic and Clinical Cooperative Research Promotion Program of Anhui Medical University (2021xkjT034) and Research Fund project of the Anhui Academy of Translational Medicine (2022zhyx-C25).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388(10055):2023–2038. doi:10.1016/S0140-6736(16)30173-8
2. Srivastava S, Rasool M. Genetics, epigenetics and autoimmunity constitute a Bermuda triangle for the pathogenesis of rheumatoid arthritis. *Life Sci*. 2024;357:123075. doi:10.1016/j.lfs.2024.123075
3. Caliogna L, Berni M, Torriani C, et al. Pathogenesis of osteoarthritis, rheumatoid arthritis, and hemophilic arthropathy: the role of angiogenesis. *Haemophilia*. 2024;30(6):1256–1264. doi:10.1111/hae.15097
4. Jang S, Kwon EJ, Lee JJ. Rheumatoid Arthritis: pathogenic Roles of Diverse Immune Cells. *Int J Mol Sci*. 2022;23(2):905. doi:10.3390/ijms23020905
5. Seyedi D, Espandar N, Hojatizadeh M, Mohammadi Y, Sadri F, Rezaei Z. Noncoding RNAs in rheumatoid arthritis: modulators of the NF-κB signaling pathway and therapeutic implications. *Front Immunol*. 2024;15:1486476. doi:10.3389/fimmu.2024.1486476
6. Tao J, Yang P, Gao M, et al. Reversing inflammatory microenvironment by a single intra-articular injection of multi-stimulus responsive lipogel to relieve rheumatoid arthritis and promote joint repair. *Mater Today Bio*. 2023;20:100622. doi:10.1016/j.mtbio.2023.100622
7. Wu CY, Yang HY, Luo SF, Lai JH. From Rheumatoid Factor to Anti-Citrullinated Protein Antibodies and Anti-Carbamylated Protein Antibodies for Diagnosis and Prognosis Prediction in Patients with Rheumatoid Arthritis. *Int J Mol Sci*. 2021;22(2):686. doi:10.3390/ijms22020686
8. Wen J, Liu J, Wan L, Wang F. Long noncoding RNA/circular RNA regulates competitive endogenous RNA networks in rheumatoid arthritis: molecular mechanisms and traditional Chinese medicine therapeutic significances. *Ann Med*. 2023;55(1):973–989. doi:10.1080/07853890.2023.2172605
9. Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495:7441):333–338. doi:10.1038/nature11928
10. Xu F, Xiao Q, Du WW, Wang S, Yang BB. CircRNA: functions, Applications and Prospects. *Biomolecules*. 2024;14(12):1503. doi:10.3390/biom14121503
11. Zhou Z, Sun B, Huang S, Zhao L. Roles of circular RNAs in immune regulation and autoimmune diseases. *Cell Death Dis*. 2019;10(7):503. doi:10.1038/s41419-019-1744-5
12. Guo G, Wang H, Ye L, et al. Hsa_circ_0000479 as a Novel Diagnostic Biomarker of Systemic Lupus Erythematosus. *Front Immunol*. 2019;10:2281. doi:10.3389/fimmu.2019.02281
13. Zheng J, Li Z, Wang T, Zhao Y, Wang Y. Microarray expression profile of circular RNAs in plasma from primary biliary cholangitis patients. *Cell Physiol Biochem*. 2017;44(4):1271–1281. doi:10.1159/000485487
14. Ouyang Q, Wu J, Jiang Z, et al. Microarray Expression Profile of Circular RNAs in Peripheral Blood Mononuclear Cells from Rheumatoid Arthritis Patients. *Cell Physiol Biochem*. 2017;42(2):651–659. doi:10.1159/000477883
15. Xue L, Wang B, Zhu J, et al. Profiling of differentially expressed circRNAs and functional prediction in peripheral blood mononuclear cells from patients with rheumatoid arthritis. *Ann Med*. 2023;55(1):175–189. doi:10.1080/07853890.2022.2156596
16. Lu H, Yang Y, Kuang D, Liu P, Yang J. Expression profile of circRNA in peripheral blood mononuclear cells of patients with rheumatoid arthritis. *BMC Med Genomics*. 2022;15(1):77. doi:10.1186/s12920-022-01225-9
17. Xiang L, Yang W, Wang F, Liu G. Circ_0083964 knockdown impedes rheumatoid arthritis progression via the miR-204-5p-dependent regulation of YY1. *J Orthop Surg Res*. 2022;17(1):558. doi:10.1186/s13018-022-03353-5
18. Zhang S, Shen Z, Chao G, et al. Circ_0004712 Silencing Suppresses the Aggressive Changes of Rheumatoid Arthritis Fibroblast-Like Synoviocytes by Targeting miR-633/TRAF6 Axis. *Biochem Genet*. 2023;61(2):521–537. doi:10.1007/s10528-022-10265-w
19. Hao J, Chen Y, Yu Y. Circular RNA circ_0008360 Inhibits the Proliferation, Migration, and Inflammation and Promotes Apoptosis of Fibroblast-Like Synoviocytes by Regulating miR-135b-5p/HDAC4 Axis in Rheumatoid Arthritis. *Inflammation*. 2022;45(1):196–211. doi:10.1007/s10753-021-01538-4
20. Wang L, Zhao H, Raman I, Yan M, Chen Q, Li QZ. Peripheral Blood Mononuclear Cell Gene Expression in Chronic Obstructive Pulmonary Disease: miRNA and mRNA Regulation. *J Inflamm Res*. 2022;15:2167–2180. doi:10.2147/JIR.S337894
21. 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;62(9):2569–2581. doi:10.1002/art.27584
22. Xia X, Tang X, Wang S. Roles of CircRNAs in Autoimmune Diseases. *Front Immunol*. 2019;10:639. doi:10.3389/fimmu.2019.00639
23. Zhai X, Zhang Y, Xin S, Cao P, Lu J. Insights Into the Involvement of Circular RNAs in Autoimmune Diseases. *Front Immunol*. 2021;12:622316. doi:10.3389/fimmu.2021.622316

24. Huang Y, Xue Q, Cheng C, et al. Circular RNA in autoimmune diseases: special emphasis on regulation mechanism in RA and SLE. *J Pharm Pharmacol*. 2023;75(3):370–384. doi:10.1093/jpp/rgac096
25. Zhang Y, Zang C, Mao M, et al. Advances in RNA therapy for the treatment of autoimmune diseases. *Autoimmun Rev*. 2025;24(4):103753. doi:10.1016/j.autrev.2025.103753
26. Tang M, Li H, Chang S, Li Y, Nie H, Li F. Dysregulated circular RNAs in rheumatoid arthritis: cellular roles and clinical prospects. *Autoimmun Rev*. 2025;24(5):103774. doi:10.1016/j.autrev.2025.103774
27. Cici D, Corrado A, Rotondo C, Cantatore FP. Wnt Signaling and Biological Therapy in Rheumatoid Arthritis and Spondyloarthritis. *Int J Mol Sci*. 2019;20(22):5552. doi:10.3390/ijms20225552
28. Chen N, Diao CY, Huang X, et al. RhoA Promotes Synovial Proliferation and Bone Erosion in Rheumatoid Arthritis through Wnt/PCP Pathway. *Mediators Inflamm*. 2023;2023:5057009. doi:10.1155/2023/5057009
29. Park YJ, Yoo SA, Kim M, Kim WU. The Role of Calcium-Calcineurin-NFAT Signaling Pathway in Health and Autoimmune Diseases. *Front Immunol*. 2020;11:195. doi:10.3389/fimmu.2020.00195
30. Ren R, Guo J, Chen Y, Zhang Y, Chen L, Xiong W. The role of Ca^{2+} /Calcineurin/NFAT signalling pathway in osteoblastogenesis. *Cell Prolif*. 2021;54(11):e13122. doi:10.1111/cpr.13122
31. Vymazal O, Bendíčková K, De Zuani M, Vlková M, Hortová-Kohoutková M, Frič J. Immunosuppression Affects Neutrophil Functions: does Calcineurin-NFAT Signaling Matter? *Front Immunol*. 2021;12:770515. doi:10.3389/fimmu.2021.770515
32. Qian K, Zheng XX, Wang C, et al. β-Sitosterol Inhibits Rheumatoid Synovial Angiogenesis Through Suppressing VEGF Signaling Pathway. *Front Pharmacol*. 2022;12:816477. doi:10.3389/fphar.2021.816477
33. Guan Y, Zhao X, Lu Y, Zhang Y, Lu Y, Wang Y. New bitongling regulates gut microbiota to predict angiogenesis in rheumatoid arthritis via the gut-joint axis: a deep neural network approach. *Front Microbiol*. 2025;16:1528865. doi:10.3389/fmicb.2025.1528865
34. Huo K, Chen T, Kong D, Zhang W, Shao J. Comprehensive analysis of circRNA expression profiles in postmenopausal women differing in bone mineral density. *Medicine*. 2024;103(16):e37813. doi:10.1097/MD.0000000000037813
35. Yan Y, Nie Y, Peng C, et al. The circular RNA hsa_circ_0001394 promotes hepatocellular carcinoma progression by targeting the miR-527/UBE2A axis. *Cell Death Discov*. 2022;8(1):81. doi:10.1038/s41420-022-00866-0
36. Longatti A, Lamb CA, Razi M, Yoshimura S, Barr FA, Tooze SA. TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. *J Cell Biol*. 2012;197(5):659–675. doi:10.1083/jcb.201111079
37. Riitano G, Recalchi S, Capozzi A, et al. The Role of Autophagy as a Trigger of Post-Translational Modifications of Proteins and Extracellular Vesicles in the Pathogenesis of Rheumatoid Arthritis. *Int J Mol Sci*. 2023;24(16):12764. doi:10.3390/ijms241612764
38. Yuan W, Peng S, Wang J, et al. Identification and characterization of circRNAs as competing endogenous RNAs for miRNA-mRNA in colorectal cancer. *PeerJ*. 2019;7:e7602. doi:10.7717/peerj.7602
39. Shi Q, Ju JG. Circ_0001998 Regulates the Proliferation, Invasion, and Apoptosis of Lung Adenocarcinoma via Sponging miR-145. *Evid Based Complement Alternat Med*. 2022;2022:6446150. doi:10.1155/2022/6446150
40. Yang T, Sun J, Wang W, et al. Hsa_circ_0006732 regulates colorectal cancer cell proliferation, invasion and EMT by miR-127-5p/RAB3D axis. *Mol Cell Biochem*. 2022;477(12):2751–2760. doi:10.1007/s11010-022-04458-5
41. Rodríguez-Trillo A, Pena C, García S, et al. ROCK inhibition with Y-27632 reduces joint inflammation and damage in serum-induced arthritis model and decreases *in vitro* osteoclastogenesis in patients with early arthritis. *Front Immunol*. 2022;13:858069. doi:10.3389/fimmu.2022.858069
42. Zheng L, Meng Y, Campbell JL, Shen B. Multiple roles of DNA2 nuclease/helicase in DNA metabolism, genome stability and human diseases. *Nucleic Acids Res*. 2020;48(1):16–35. doi:10.1093/nar/gkz1101
43. Zhao J, Xu L, Wei K, et al. Identification of clinical characteristics biomarkers for rheumatoid arthritis through targeted DNA methylation sequencing. *Int Immunopharmacol*. 2024;131:111860. doi:10.1016/j.intimp.2024.111860
44. Geng S, Hu B, Guan Y, et al. Advances of the multifaceted functions of PSTPIP2 in inflammatory diseases. *Front Immunol*. 2024;15:1492878. doi:10.3389/fimmu
45. Yao Y, Cai X, Zhang M, et al. PSTPIP2 regulates synovial macrophages polarization and dynamics via ERβ in the joint microenvironment. *Arthritis Res Ther*. 2022;24(1):247. doi:10.1186/s13075-022-02939-y
46. Miao J, Zhang J, Huang X, Wu N, Wu D, Shen M. *NLRP12*-associated autoinflammatory disease in Chinese adult patients: a single-centre study. *RMD Open*. 2023;9(4):e003598. doi:10.1136/rmdopen-2023-003598
47. Huang Y, Xu L, Yang Q, et al. NLRP12 c.1382dup promotes the development of Crohn's disease through the ERK/NLRP3/ IL-1β pathway. *Gene*. 2024;931:148855. doi:10.1016/j.gene.2024.148855
48. Tsao YP, Tseng FY, Chao CW, et al. NLRP12 is an innate immune checkpoint for repressing IFN signatures and attenuating lupus nephritis progression. *J Clin Invest*. 2023;133(3):e157272. doi:10.1172/JCI157272
49. Zhang X, Nan H, Guo J, Liu J. NLRP12 reduces proliferation and inflammation of rheumatoid arthritis fibroblast-like synoviocytes by regulating the NF-κB and MAPK pathways. *Eur Cytokine Netw*. 2021;32(2):15–22. doi:10.1684/ecn.2021.0465