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Circulating exo-miRNA-27a-5p is a novel biomarker of the tofacitinib treatment response in rheumatoid arthritis

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Abstract

Background Effective biological markers able to monitor the response of Janus kinase inhibitor (JAKi) are lacking. Exosomal microRNAs (exomiRNAs) can alter their expression during treatment and are ideal biomarkers for therapeutic interventions. In this study, we explored potential biomarkers for monitoring tofacitinib treatment response in patients with RA.

Methods Peripheral blood mononuclear cells (PBMCs) were collected from 35 healthy controls (HCs) and 74 patients with methotrexate (MTX)-resistant new-onset RA. We analyzed the profiles of exomiRNAs using next-generation sequencing (NGS) and verified them using quantitative real-time polymerase chain reaction (qRT-PCR). The functional roles of the selected exomiRNAs were analyzed using bioinformatics tools. Potential exomiRNAs were validated in MTX-resistant RA patients treated with tofacitinib for 3 months.

Results Fifty-six differentially expressed exomiRNAs were identified. High expressions of the exo-(miR-548ah-3p, miR-378 g, miR-27a-5p, and miR-30c-2-3p) were validated by qRT-PCR. Enrichment analysis indicated that these exomiRNAs may regulate immune cells and mediate immune responses. Exo-miR-27a-5p levels significantly decreased after tofacitinib treatment ($p < 0.0001$) and showed a strong correlation with the DAS28, RF and ESR. Receiver operating characteristic curve analysis showed that changes in the expression levels of exo-miR-27a-5p were significantly correlated with tofacitinib therapy (AUC = 0.92, $p < 0.0001$).

Conclusions This study suggests that circulating exo-miR-27a-5p is a novel non-invasive biomarker to monitor the response to tofacitinib treatment.

Key points

- JAKi is frequently used to treat rheumatoid arthritis; however, no effective biological markers are available to monitor the treatment response.
- Exo-miR-27a-5p is a novel non-invasive biomarker for monitoring the response to tofacitinib treatment.

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Keywords Rheumatoid arthritis, Circulating exosomes, MiRNAs, Biomarker, Tofacitinib

Background

Rheumatoid arthritis (RA) is a systemic rheumatic disease with persistent joint destruction as its main clinical manifestation; its prevalence in China is approximately 0.28% [1]. Early diagnosis, standardized treatment, and treatment-to-target (T2T) are the clinical treatment approaches for patients with RA. Sustained intensive treatment is a better approach for achieving clinical remission and better clinical outcomes [2]. However, rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPAs) are not satisfactory markers for clinical use and are neither diagnostic biomarkers nor good predictors of responses to disease-modifying antirheumatic drugs (DMARDs) [3–5]. These findings indicate the need to identify new non-invasive biomarkers to improve personalized therapeutic decisions.

In recent years, growing evidence has shown that microRNAs (miRNAs) play key roles in RA, including genetic susceptibility, immune inflammation, changes in synovial pathology, and bone destruction [6, 7]. MiR-22 is associated with joint damage in patients with early RA, indicating that it is a circulating diagnostic biomarker [8]. The miR-224, miR-760, miR-483-5p, miR-378, and miR-375 levels are positively correlated with the disease activity score (DAS)28 and may be prognostic biomarkers [9]. Furthermore, some miRNAs can predict the response to TNF-a inhibitors, such as the levels of miR-99a, miR-143 miR-23a, and miR-197, which are associated with the response to Adalimumab (ADA) and Etanercept (ETN), respectively [10].

Exosomes are carriers of miRNAs, 30–150 nm in diameter, and are widely detected in plasma, serum, urine, and other body fluids; they also play a role in mediating communication between different tissues and cells, making them ideal biomarkers [11–13]. In recent studies, several circulating exomiRNAs have been identified as diagnostic biomarkers of RA, such as exo-miR-1915-3p, exo-miR-451a, and exo-miR-25-3p [14, 15]. A few studies have suggested that exo-miR-122-5p, exo-miR-155-5p [16], miR-1298-5p [17], and miR-19b [18] may act as novel biomarkers of the efficacy of tofacitinib. Although circulating exomiRNAs have been established as RA biomarkers, their role in predicting drug efficacy remains unclear. In this study, we explored circulating exomiRNAs as novel biomarkers of the therapeutic efficacy of tofacinib and analyzed their biological functions.

Methods

Study design

Seventy-four patients with RA who met the criteria for the American College of Rheumatology/European League Against Rheumatism classification (2010) and sixty-one healthy controls (HCs) were recruited from volunteers during the same period. Patients with RA remained in high disease activity after methotrexate (MTX) treatment for more than 3 months and had a disease duration of less than 1 year. Patients were divided into four groups: sequencing, filtering, validation, and treatment. In the sequencing group, samples from HCs ($n=4$) and patients with RA ($n=4$) were analyzed using next-generation sequencing (NGS). In the filter group, samples from HCs ($n=5$) and patients with RA ($n=5$) were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) to screen for exomiRNAs. HCs ($n=26$), and RA ($n=33$) samples from the validation group were used to confirm exomiRNAs. In the treatment group, RA patients ($n=32$) with a previous failure of MTX monotherapy would receive tofacitinib treatment for an additional 3 months. The clinical characteristics of the participants are shown in Table 1.

Sample collection and exosome isolation

Fresh blood samples were obtained from participants in EDTA collecting tubes (BD, USA). The PBMCs were isolated by the EasySep™ Direct Human PBMC Isolation Kit (STEMCELL, Beijing, China) following the protocol. Exosomes were isolated using different centrifugation procedures, as described previously [19].

Circulating exosome identification

We used three methods to identify exosomes: nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and Western blotting identification of protein markers. All procedures were performed as described previously [20]. Briefly, exosomes were dropped onto a copper grid for 1 min and phosphotungstic acid was added for 30 s. Images were captured at 80 kV using TEM (Hitachi, H7650, Japan). The particle size and concentration of the exosomes were measured by NTA (Particle Metrix, Germany). Exosome markers, including CD9 (1:1000, ab307085, Abcam), CD63 (1:2000, MA5-35208, Thermo Fisher), and HSP70 (1:1000, 4872, Cell Signaling Technology) were identified by Western blotting.

Isolation and sequencing of ExomiRNAs

Total exomiRNAs were extracted by RNAzol® RT RNA isolation reagent (GeneCopoeia, QP020, USA) according to the manufacturer's protocol. ExomiRNAs were sequenced using a HiSeq 2500 (Illumina, USA) at RiboBio Co., Ltd. (RiboBio, China). Differentially expressed exomiRNAs were filtered using the following criteria

Table 1 Basic clinical characteristics of patients with rheumatoid arthritis and healthy controls

Clinical variables	Sequencing group		Filter group		Validation group		Treatment group
	HC (4)	RA (4)	HC (5)	RA (5)	HC (26)	RA (33)	RA(32)
Age (years)	44±11.5	55.5±4.4	49±18.8	56.8±14.1	54.9±9.1	57.5±8.5	54.8±11.3
Male/Female (n)	0/4	0/4	1/4	1/4	5/21	7/26	7/25
RF (IU/mL)	NA	166.6 (152.4–179.6)	NA	161.3 (145–185.4)	NA	172 (22.4–394)	124.2 (3.5–203.6)
ACPA (RU/mL)	NA	1842 (1600–2000)	NA	1289 (157.2–2000)	NA	1053 (117–1951)	647.9 (110.6–2000)
CRP (mg/L)	NA	49.3 (9.2–147.8)	NA	15.75 (1.30–46.5)	NA	23.3 (6.2–53)	27.5 (1.2–92)
ESR (mm/h)	NA	82 (70.8–98)	NA	88.8 (57–104)	NA	69.7 (32–110)	62 (11–120)
DAS28	NA	6±0.5	NA	5.4±0.6	NA	5.9±0.5	5±0.9

(|log2FC| > 3, adj. p-value < 0.05) based on the false discovery rate (FDR), as shown in the Supplementary Table 1.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The expression of circulating exomiRNAs was measured by qRT−PCR using the All-in-One™ miRNA qPCR Kit (GeneCopoeia, QP010, USA), as described previously [21]. Cel-miR-39-3p (miRB0000010-3-1; RiboBio, China) was used as an external control. The expression of exomiRNAs was determined in three replicates and calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used are listed in Supplementary Table 2.

Prediction of the potential target gene of ExomiRNAs

To investigate the potential regulatory genes of exomiRNAs, we used three online databases, including TargetScan, miRDB, and mirDIP. The results extracted from the three databases were compared to obtain a set of potential regulatory target genes for exomiRNAs. We analyzed the data from three microarray datasets (GSE55235, GSE55457, and GSE77298) from the Gene Expression Omnibus (GEO) database, as shown in Online Resource 1, Supplementary Table 3. Differentially expressed genes (DEGs) in RA were evaluated using a conservative threshold (|log2FC| > 0.5, adj. p-value < 0.05). Finally, we searched for genes in public databases, including CTD, DisGeNET, DrugBank, GeneCards, OMIM, and TTD. The "marker/mechanism and therapeutic" genes for direct evidence were selected from the CTD database. In the DisGeNET and GeneCards databases, genes with Score_gda and relevance scores higher than the average values were selected, and a defined Entrez Gene ID was required in the OMIM database. Potential regulatory target genes for exomiRNAs were obtained by comparing the prediction target sets with three GEO microarray datasets and six public databases.

Functional enrichment analysis

We used Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) to annotate the potential targets of exomiRNAs through the clusterProfiler package (v.4.7.1). The selection criteria were a p-value < 0.05 and a q-value < 0.05. Based on the enrichment analysis results, a data file was then established to construct the interaction network between potential regulated targets of exomiRNAs and pathways using the Cytoscape software.

Construction of the protein–protein interaction (PPI) network

The STRING database (v.11.0) was used to construct an interaction network of potential exomiRNA targets in RA. Parameter settings included *Homo sapiens* as the organism and a combined score > 0.4. Molecular complex detection (MCODE) in Cytoscape (v.3.7.1) was used to analyze the network module, and the protein–protein interaction (PPI) network was constructed using Gephi (v.0.9.2).

Statistical analysis

Data are presented as the median (interquartile range) or mean ± standard deviation (SD). The Wilcoxon signed rank test for matched pairs and Mann–Whitney U test were used to calculate differences between patients with RA and HCs. The false discovery rate (FDR) was calculated using the BioLadder modules in the proteome data analysis framework online (https://www.bioladder.cn/web/#/pro/index). To evaluate the sensitivity and specificity of circulating exomiRNAs as biomarkers for Janus kinase inhibitors (JAKis), the Wilson/Brown method was used in ROC curve analysis via the MedCalc statistical software. Statistical analyses were performed using R v.4.0.2 and Prism v.9.5 (GraphPad, San Diego, CA, USA). P-values < 0.05 were considered statistically significant.

Results

Characterization of circulating exosomes

Circulating exosomes isolated from the HCs and participants with RA were identified using TEM, NTA, and Western blotting. According to the TEM and NTA results, the exosome size ranged from 30 to 150 nm in the HC and RA groups (Fig. 1a–d). Specific markers CD9, CD63, and HSP70 were detected by Western blotting and

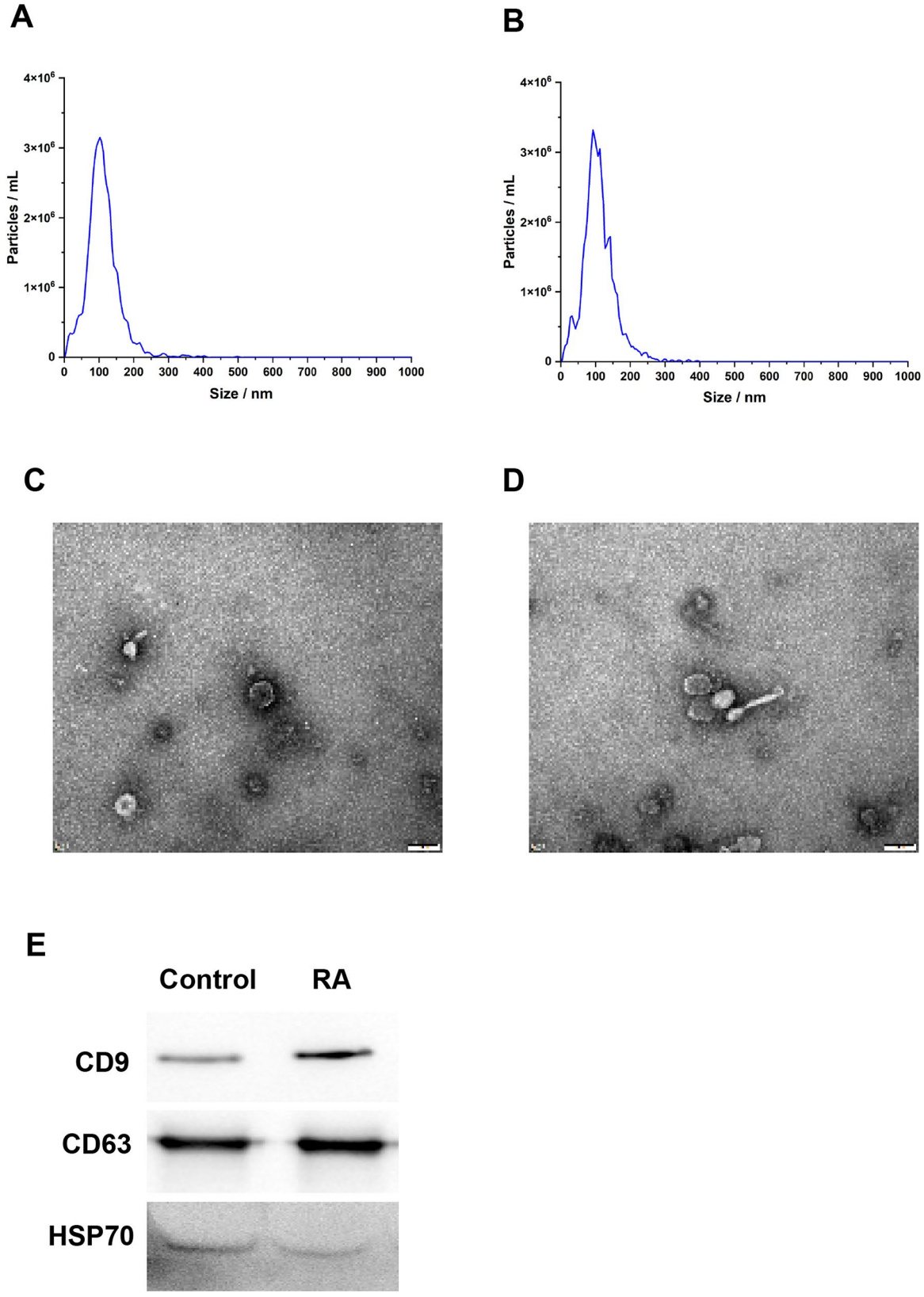

Fig. 1 Identification of circulating exosomes. (**a**, **b**) Size and concentration of circulating exosomes from patients with rheumatoid arthritis (RA) and healthy controls (HCs) analyzed by nanoparticle tracking analysis (NTA), respectively; (**c**, **d**) Circulating exosomes of patients with RA and HCs verified by transmission electron microscopy (TEM), respectively; scale bar: 200 nm. (**e**) Specific exosome markers CD9, CD63 and HSP70 analyzed by Western blotting

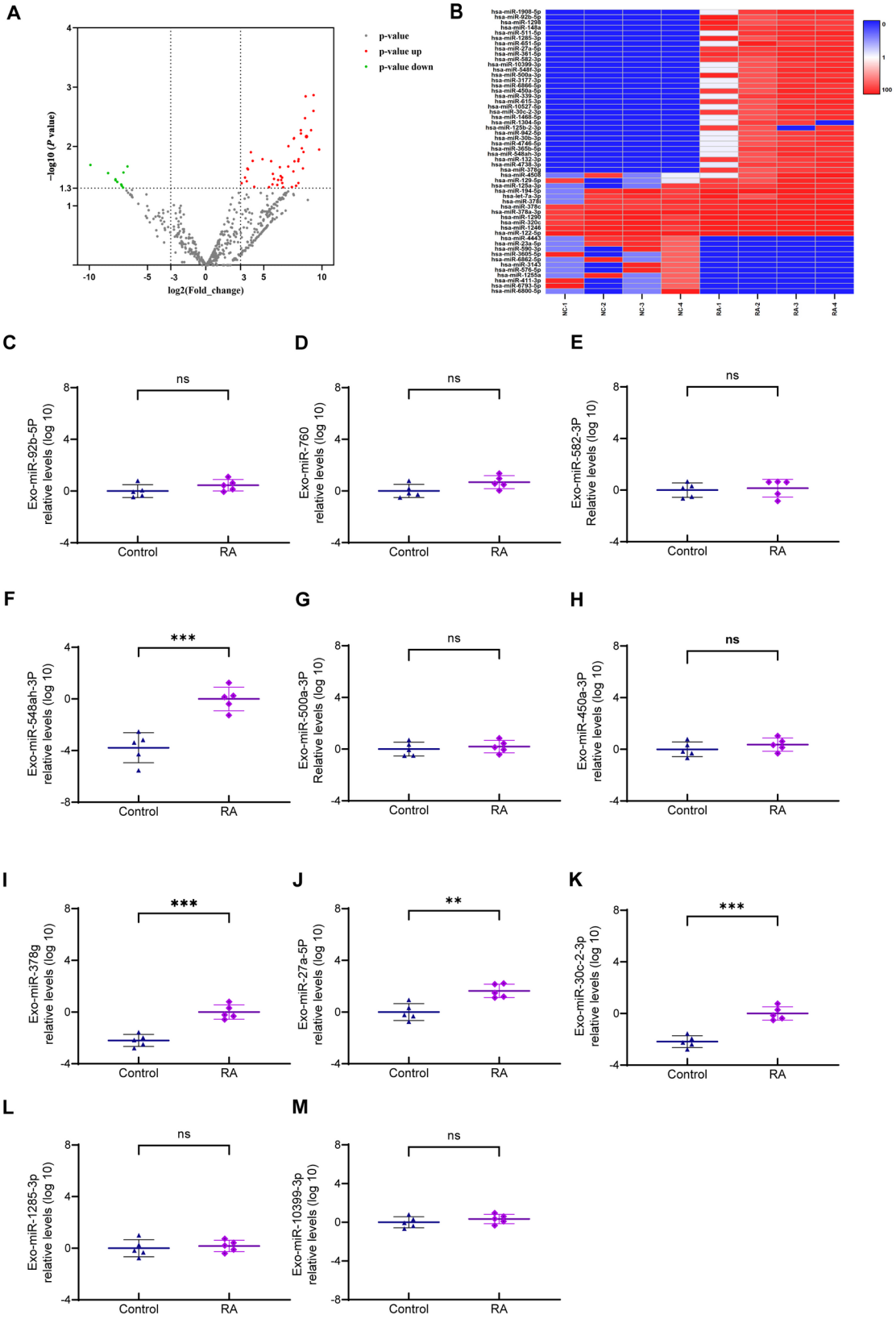

Fig. 2 Screening of differentially expressed circulating exomiRNAs in patients with RA and HCs. (**a**) Volcano map of differentially expressed circulating exomiRNAs in the sequencing group; Green: downregulated exomiRNAs in the sequencing group; Gray: not significant. (**b**) Heatmap of differentially expressed circulating exomiRNAs. (c–m) qRT-PCR detected the expression of 11 candidate circulating exomiRNAs in the selected group. The values are normalized to Cel-miRNA-39-3p and shown on a log10 scale on the y-axis. All data are represented as the mean ± SD. ***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$

were clearly observed in circulating exosomes of both groups (Fig. 1e).

Differential expression of circulating ExomiRNAs from patients with RA and HCs

We evaluated the expression of exomiRNAs using NGS in PBMCs from patients with RA and HCs. The levels of 45 exomiRNAs were significantly elevated, whereas those of 11 exomiRNAs were decreased (Fig. 2a, b). Based on the *p*-value and fold changes, we selected 11 exomiRNAs (exo-miR-92b-5p, exo-miR-582-3p, exo-miR-548ah-3p, exo-miR-500a-3p, exo-miR-450a-3p, exo-miR-378 g, exo-miR-27a-5p, exo- miR-1285-3p, exo-miR-10399-3p, exo-miR-30c-2-3p, and exo-miR-760) for preliminary screening in the filtered group using qRT-PCR (Fig. 2c–m).

The expression levels of exo-miR-548ah-3p, exo-miR-378 g, exo-miR-27a-5p, and exo-miR-30c-2-3p) in the PBMCs of patients with RA were significantly different from those of the HCs. Similar findings were observed in the qRT-PCR results of the validation group (Fig. 3a–d). The ROC areas of exo-miR-27a-5p, exo-miR-30c-2-3p, exo-miR-548ah-3p, exo-miR-378 g were 0.79 (95% CI: 0.67– 0.91), 0.80 (95% CI: 0.68–0.91), 0.82 (95% CI: 0.71– 0.93), and 0.74 (95% CI: 0.59–0.88), respectively (Fig. 3e). We analyzed the profiles of four potential exo-miRNAs as a whole to distinguish the healthy controls and RA patients. The results showed that the estimated AUC was 0.97 (95%CI: 0.89–0.99, $p < 0.0001$, cut off value: 0.69, YI: 0.81, sensitivity: 84.85%, specificity: 96.15%, as shown in Fig. 3e).

Prediction and screening for ExomiRNA targets

Based on online databases, we predicted that exo-miR-27a-5p, exo-miR-30c-2-3p, exo-miR-378 g, and exo-miR-548ah-3p had 186, 78, 358, and 48 potential target genes, respectively. A total of 631 target genes regulated by the four exomiRNAs were identified after merging the data (see Online Resource 1, Supplementary Fig. 1a–d). Furthermore, we analyzed DEGs from the GEO database, and 2757 DEGs associated with RA were obtained after merging the data (see Online Resource 1, Supplementary Fig. 2a–f). A total of 106 potential target genes of the four exomiRNAs were obtained after merging the profiles of three-GEO, and -online databases. Furthermore, we selected and identified 1731 RA-related genes by merging them from six public databases (CTD, DisGeNet, DrugBank, GeneCards, OMIM, and TTD) and 190, 472, 586, 841, 314, and 130 genes, respectively (see Online Resource 1, Supplementary Fig. 2g). After combining the results of the above two methods, 133 target genes of the exomiRNAs were identified (see Online Resource 1, Supplementary Fig. 2h). Potential target genes were subjected to functional enrichment analysis.

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The clusterProfiler R package was used to analyze 133 target genes regulated by circulating exomiRNAs. The results from the gene ontology (GO) analysis showed that these target genes were mainly enriched in 169 biological processes, including regulation of hemopoiesis (GO:1903706), lymphocyte differentiation (GO:0030098), mononuclear cell differentiation (GO:1903131), myeloid cell differentiation (GO:0030099), gland development (GO:0048732), embryonic organ development (GO:0048568), cell–cell adhesion regulation (GO:0022407), alveolar-type cell migration (GO:0001667), regulation of leukocyte differentiation (GO:1902105), and B-cell activation (GO:0042113). Two molecular functions (MFs) were also enriched: protein serine/threonine kinase activity (GO:0004674) and protein serine kinase activity (GO:0106310) (Fig. 4a). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that the 133 RA-related genes regulated by exomiRNAs were enriched in 84 pathways, including in the human papillomavirus infection (hsa05165), influenza A (hsa05164), Alzheimer's disease (hsa05010), JAK/STAT signaling pathway (hsa04630), Kaposi sarcoma-associated herpesvirus infection (hsa05167), Epstein–Barr virus infection (hsa05169), and proteoglycans in cancer (hsa05205) (Fig. 4b). The enrichment network revealed the relationship between potential exomiRNA target genes and pathways (Fig. 4c).

PPI network analysis of the hub genes of ExomiRNAs

The 133 target genes regulated by exomiRNAs were imported into the STRING database and a PPI network was constructed. Seven genes showed high degree values: *AKT1* (34), *FN1* (23), *STAT1* (14), *IL2* (12), *CASP8* (10), *HSPA4* (10), and *PIK3CA* (10) (Fig. 4d). Furthermore, the MCODE analysis revealed that a submodule was present in the PPI network, which included *AKT1*, *FN1*, *STAT1*, *IL2*, *CASP8*, *HSPA4*, *ATG7*, *CCR5*, and *PTPN2* (Fig. 4e). Interestingly, five of nine genes had higher degree values (degree ≥ 10) in the PPI network. This module is a key submodule of the exosome-derived miRNA regulatory target gene PPI network (Fig. 4f).

Impact of Tofacitinib therapy on the expression of potential ExomiRNAs

Bioinformatics analysis indicated a possible association between circulating exo-(miR-27a-5p, miR-30c-2-3p, miR-378 g, and miR-548ah-3p) and the JAK/STAT pathway. Consequently, tofacitinib was selected as the therapeutic drug, and alterations in exosomal miRNAs in patients with RA were monitored before and after treatment. The results indicated a significant decrease in exo-miR-27a-5p levels following tofacitinib treatment

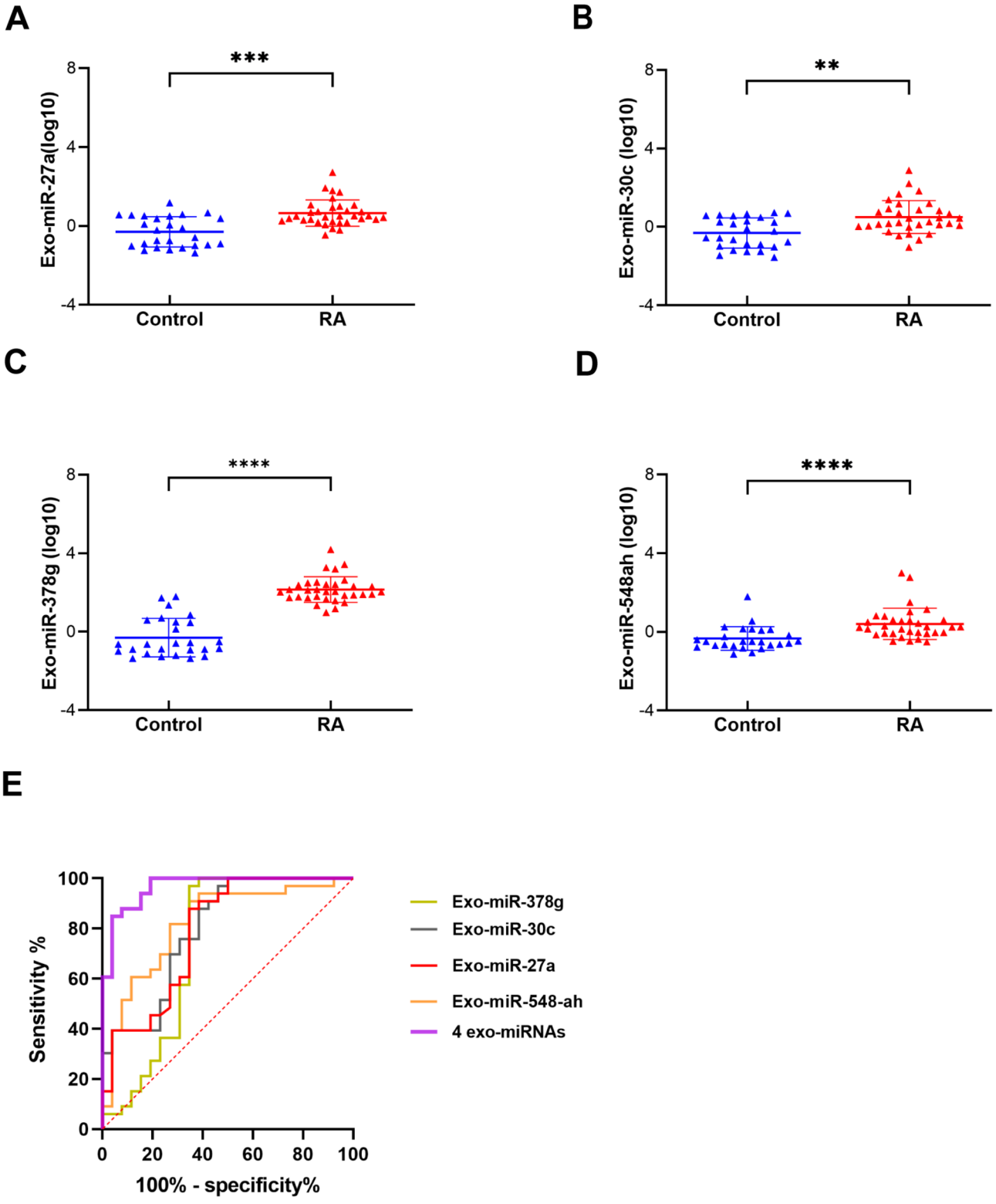

Fig. 3 Validation of candidate targets of circulating exomiRNAs. (**a**–**d**) Relative levels of four circulating exomiRNAs in the validation group. (**e**) Receiver operating characteristic (ROC) curve of four circulating exomiRNAs for RA. The values are normalized to Cel-miRNA-39-3p and shown on a log10 scale on the y-axis. All data are shown as mean ± SD. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

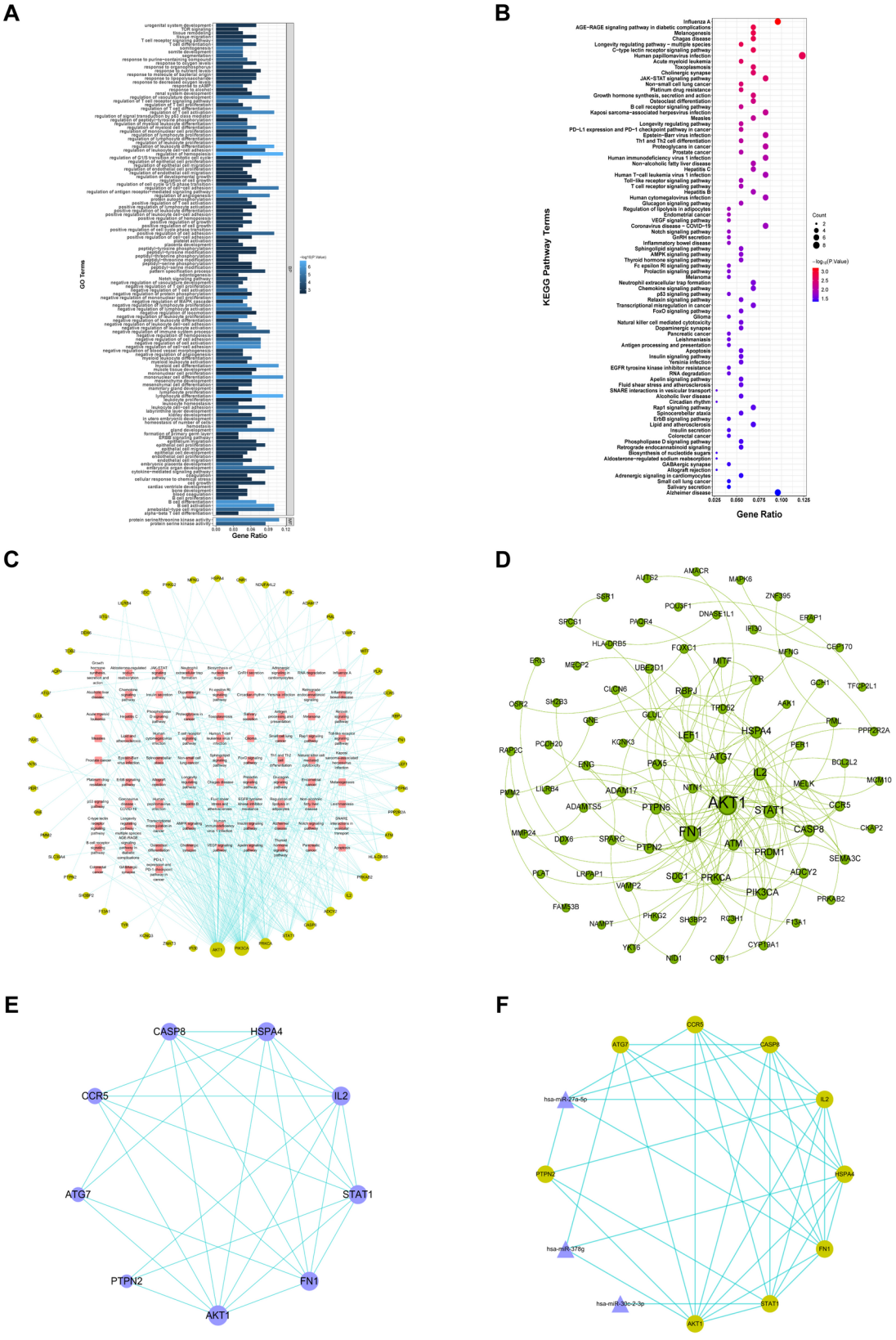

Fig. 4 (See legend on next page.)

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Fig. 4 Enrichment analyses of the biological functions of exomiRNAs. (**a**) Gene ontology (GO) annotation of the biological processes (BPs) and molecular functions (MFs) for targets of exomiRNAs. (**b**) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of possible signaling pathways of exomiRNAs. (**c**) Enrichment analysis network between target genes of exomiRNAs and pathways. (**d**) PPI network showing the 133 exomiRNA targets. (**e**) MCODE analysis showing the network of the 9 hub genes. (**f**) Regulatory network between exomiRNAs and 9 hub genes. The brown circles represent hub genes and the blue triangles represent exomiRNAs

($p < 0.001$), but without statistical variance when compared with HCs (Fig. 5a). the expression levels of exo-miR-548ah-3p, exo-miR-378 g, and exo-miR-30c-2-3p were not significantly different ($p > 0.05$) after JAKi therapy (as shown in Online Resource 1, Supplementary Fig. 3a–c). The altered expression of exo-miR-27a-5p was strongly correlated with DAS28, RF, and ESR after tofacitinib therapy (Fig. 5b–d). However, the remaining three exomiRNAs were not correlated with RF or ESR ($p > 0.05$); only exo-miR-378 g was correlated with the DAS28 score ($p = 0.001$, see Online Resource 1, Supplementary Fig. 4a–c). The ROC curve was performed to evaluate the value of exosomal miR-27a-5p expression levels in predicting therapeutic response to JAK inhibitor (JAKi) therapy. The estimated AUC was 0.92 (95% CI: 0.82–0.97, $p < 0.0001$, cut off value: 0.93, YI: 0.75, sensitivity: 90.62%, specificity: 84.37%, shown in Fig. 5e). The results of ROC curve indicated that changes in the exo-miR-27a-5p expression levels were significantly correlated with JAKi therapy outcomes.

Discussion

Tofacitinib is a sustained intensive DMARD for clinical RA treatment with good efficacy and safety. Evidence suggests that the downregulation of tumor necrosis factor-α (TNFα), interleukin- 6 (IL-6), C-X-C motif chemokine ligand 1(CXCL1), matrix metalloproteinase-1(MMP-1), IL-17 A, and IL-17 C mRNA after tofacitinib therapy is associated with clinical remission of RA [22–24], indicating that they can be biomarkers for assessing the response to JAKi therapy. The lower the multi-biomarker disease activity (MBDA) scores of patients with RA receiving tofacitinib treatment, the better their clinical outcomes [25], which indicates that the musculoskeletal ultrasound (MSUS) can be a predictive tool for the response to JAKi. However, the efficacy of JAKi therapy is difficult to assess and predict.

In our research, the age of the HCs was lower than that of RA patients in the group of sequencing and filtering. Recently, some evidence that RA might be related to age has been reported [26]. The younger patients with RA exhibited higher clinical remission rates and lower radiological progression than older patients [27]. However, the relationship between age and RA remained controversial. The prevalence of ACPA-negative among older populations with RA was higher, whereas there was no significant correlation with age in the ACPA-positive subgroups [28]. Furthermore, the clinical remission timelines showed no statistically significant differences between older and younger patients with RA. These findings did not support the definitive relationship between age and RA. Consequently, age is unlikely to constitute a confounding variable and should not affect the outcomes of our experiment.

In this study, 56 differentially expressed exomiRNAs were identified by NGS sequencing analysis, and exo-(miR-548ah-3p, miR-378 g, miR-27a-5p, and miR-30c-2-3p) were elevated in the PBMCs of MTX-resistance patients with RA. Their biological functions were found to regulate the activation and signal transduction of immune cells and the immune–inflammatory response via the JAK/STAT signaling pathway, the B-cell receptor pathway, the Toll-like receptor pathway, Fc gamma R-mediated phagocytosis, and the apoptosis-related pathway. In further research, we observed the changes in circulating exosomes from MTX-resistant patients with RA before and after three months of JAKi treatment to evaluate whether these exosomes could serve as predictive efficacy markers of JAKi.

Previous studies have shown that exo-(miR-548ah-3p, miR-378 g, and miR-30c-2-3p) may be associated with the immune response and inflammation. MiR-378 g is one of the least studied members of the miR-378 family, and its function in RA has not yet been studied. Researchers found that miR-378 g promotes osteogenic differentiation by targeting nicotinamide N-methyltransferase (NNMT) during bone metabolism [29]. In addition, miR-378 g regulates the Treg/Th17 imbalance via CCL5 [30], and exo-miR-378 g regulates macrophage polarization, T-cell activation, and inflammatory cytokine transcription [31]. Numerous studies have found that miR-30c-2-3p regulated the activity of mitogen-activated protein kinase (MAPK) [32] and the NF-κB pathway [33] involved in inflammatory processes, apoptosis, and cell proliferation. Exo-miR-30c-2-3p can be captured and is highly expressed in plasma exosomes, serving as a biomarker for neurodegenerative diseases [34]. Some studies have shown that exo-miR-30c-2-3p mediates the expression of AKT1S1 and EIF4B, which play significant roles in PI3K/AKT signaling [35]. In this study, these exo-(miR-548ah-3p, miR-378 g, and miR-30c-2-3p) showed high expression only in the peripheral blood of patients with RA, suggesting poor correlation with clinical indicators and the prediction efficacy of JAKi.

The involvement of miR-27a-5p in the progression of RA has not been reported previously. However, it is

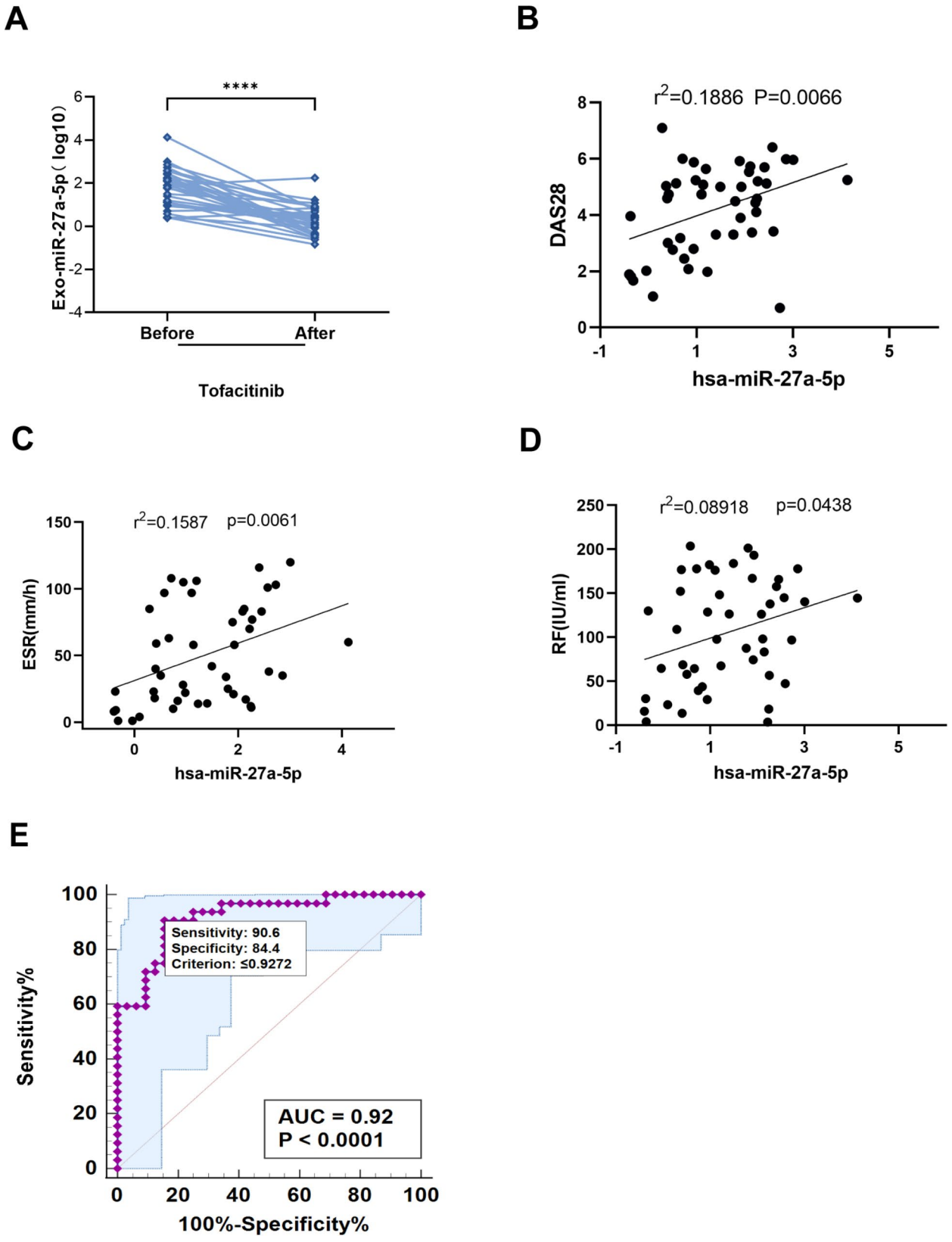

Fig. 5 Impact of tofacitinib therapy on the expression of potential exomiRNAs. (**a**) Levels of exo-miR-27a-5p before and after tofacitinib therapy. (**b**–**d**) Correlation analysis of exo-miR-27a-5p expression with DAS28, ESR, and RF before and after tofacitinib treatment. (**e**) ROC analysis of the sensitivity and specificity between changes in the expression of exo-miR-27a-5p and tofacitinib therapy. Values are normalized to CelmiRNA-39-3p and are shown on a log10 scale on the *y*-axis. All data are shown as the mean ± SD. ****$p < 0.0001$, ***$p < 0.001$, ** $p < 0.01$, * $p < 0.05$

encapsulated in extracellular vesicles and participates in apoptosis and inflammation [36, 37]. Some studies have shown that miR-27a-5p also inhibits M2-like macrophage polarization through the Ppm1l and PI3K/Akt pathways [38, 39]. These studies indicate that exo-miR-27a-5p plays an essential role in the immune and inflammatory processes of different diseases and is an essential pathogenic mechanisms of RA. Based on the PPI network, protein tyrosine phosphatase N2 (PTPN2) may be a potential target of miR-27a-5p, which is a negative regulator of the T-cell receptor (TCR) and JAK/STAT signaling pathways [40]. Furthermore, low expression of PTPN2 accelerates IL-6 production and enhances the T-cell-mediated immune response [41, 42]. Based on these findings, we concluded that the JAK/STAT pathway may play a key role in the biological functions of miR-27a-5p, demonstrating its potential as a biomarker of JAKi therapy.

In conclusion, this study demonstrated that exo-miR-548ah-3p, exo-miR-378 g, exo-miR-27a-5p, and exo-miR-30c-2-3p expressions were elevated in the PBMCs of MTX-resistant RA patients. Furthermore, circulating exo-miR-27a-5p may act as a novel biomarker in monitor clinical response of tofacitinib treatment. However, there are some limitations in our study, such as the limited sample size and lack of confirmation using histological samples, which should be incorporated into future research plans.

Abbreviations

ACPA	Anti-citrullinated peptide antibodies
CRP	C-reactive protein
DAS28	Disease activity score (DAS)28
DEGs	Differentially expressed genes
DMARDs	Disease-modifying antirheumatic drugs
ESR	Erythrocyte sedimentation rate
Exo-miRNAs	Exosomal microRNAs
GO	Gene ontology
HCs	Healthy controls
JAKi	Janus kinase inhibitor
KEGG	Kyoto Encyclopedia of Genes and Genomes
MCODE	Molecular complex detection
MIRNAs	MicroRNAs
MTX	Methotrexate
NA	Not applicable
NGS	Next-generation sequencing
PBMCs	Peripheral blood mononuclear cells
PPI	Protein–protein interaction
qRT-PCR	Quantitative real-time polymerase chain reaction
RA	Rheumatoid arthritis
RF	Rheumatoid factor
ROC	Receiver operating characteristic curve
T2T	Treatment-to-target

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41927-025-00502-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions
Design and revision: F.L.(Fuqun Liu); Writing: J.Z.(Jiwei Zhao); data curation: T.Z. (Tianjun Zhu); Bioinformatics: J.S.(Jijia SUN); resources: Q. L.

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Data availability
The datasets of miRNAs-seq generated and analysed during the current study are available in the PRJNA1204188 repository, [https://dataview.ncbi.nlm.nih.gov/object/PRJNA1204188?reviewer=mhrovmvv0har4ap6h7dv6fckn6].

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Lishui District Hospital of Traditional Chinese Medicine in accordance with the Declaration of Helsinki (1989) (No. 2021KYLW007). Clinical trial number was not applicable. Blood samples were collected without any medical intervention. At the beginning of this study, each participant completed informed consent forms. The privacy of patients was protected throughout this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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