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In silico evaluation of hsa-miR-125a-5p and hsa-miR-125b-5p as potential biomarkers for monitoring acupuncture treatment in rheumatoid arthritis

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ABSTRACT

Objective To perform an *in silico* bioinformatics analysis to identify differentially expressed microRNAs (miRNAs) implicated in rheumatoid arthritis (RA) pathogenesis and evaluate their potential as biomarkers for assessing therapeutic efficacy and monitoring acupuncture treatment.

Methods miRNA microarray data (CEL and TXT formats) were acquired from human and murine RA models, with the latter undergoing acupuncture treatment. Data were normalized using the robust multi-array average (RMA) method and analyzed for differential expression. Differential expression analysis identified miRNAs through a comparative analysis of RA human tissues, acupuncture-treated murine RA models, and a bibliographic search for miRNAs implicated in RA pathogenesis and acupuncture treatment. Bioinformatics analysis was performed to identify potential target genes for each miRNA and signaling pathways via search tools for the Retrieval of Interacting Genes/Proteins (STRING) and ShinyGO. Gene-drug interaction analysis was performed through Drug-Gene Interaction Database (DGIdb) screening. Interaction networks were constructed with the Cytoscape v3.10.3 software.

Results The hsa-miR-125a-5p and hsa-miR-125b-5p were identified as potential biomarkers associated with RA pathogenesis, presenting 468 and 455 target genes, respectively. These genes were enriched in 20 signaling pathways, including Janus kinasa-signal transducer and activator of transcription (JAK-STAT), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase-protein kinase B (PI3K-Akt), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which have been associated with RA pathogenesis and progression. Drug-gene interaction networks revealed that 22 genes were significantly associated with 58 RA treatment drugs, among which 13 genes interacted with

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members of the hsa-miR-125 family.

Conclusion The hsa-miR-125a-5p and hsa-miR-125b-5p demonstrate critical regulatory role in RA pathogenesis by modulating signaling pathways, including JAK-STAT, MAPK, PI3K-Akt, and NF-κB. Our findings show that the hsa-miR-125a-5p and hsa-miR-125b-5p exhibit differential expression patterns in response to pharmacological intervention in various diseases, including RA management. This suggests their potential roles as biomarkers for monitoring acupuncture treatment. Although existing evidence indicates that acupuncture can modify miRNA expression profiles, rigorous validation through biological models remains essential to confirm these results.

1 Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by immune-mediated synovial inflammation, leading to progressive joint destruction and functional impairment. The disease is defined by the presence of autoantibodies against IgG (rheumatoid factor) and citrullinated proteins (anti-citrullinated protein antibodies), which serve as diagnostic biomarkers and contributors to pathogenesis [1, 2]. While these antibodies are a key feature of RA (seropositive RA), some patients are negative for these antibodies (seronegative RA), highlighting disease heterogeneity and the interplay between environmental triggers and genetic susceptibility [3]. RA, as a chronic inflammatory joint disease, affects 0.5% – 1.0% of the global population, showing a female preponderance (male : female ratio of 1 : 2 – 1 : 3) and bimodal age distribution with peak incidence at the age of 60 years. Temporal and geographic variations in incidence are influenced by genetic polymorphisms and environmental exposures, underscoring the multifactorial etiology of this debilitating condition [4].

RA pathophysiology is characterized by chronic inflammation of the synovial membrane, driven by dysregulated interactions among T lymphocytes, B lymphocytes, and monocytes, which can lead to the destruction of articular cartilage and juxta-articular bone. This immune dysregulation promotes synovial hyperplasia, with fibroblast-like synoviocytes and macrophages resulting in a proliferative synovial tissue, known as pannus, which invades the periarticular bone at the cartilage-bone junction and articular cartilage. Some molecules, such as receptor activator of nuclear factor kappa-B ligand (RANKL), prostaglandins, and matrix metalloproteinases, are induced by proinflammatory cytokines [e.g., tumor necrosis factor (TNF) and interleukin (IL)-6], regulating the disease signs and symptoms, such as pain, inflammation, and cartilage and bone degradation [5, 6].

Acupuncture is effective in treating RA via multiple mechanisms. Vagus nerve activation during acupuncture stimulates dopamine production in the adrenal medulla, which may contribute to systemic anti-inflammatory responses and the attenuation of sepsis-related pathways. Clinically, this intervention significantly reduces joints pain, improves overall patient scores, and enhances long-term outcomes. Mechanistically, acupuncture downregulates proinflammatory cytokines [e.g., IL-6, TNF-α, and vascular endothelial growth factor (VEGF)] in serum and synovial tissues, while upregulating anti-inflammatory cytokines, such as IL-4 and IL-10. However, the precise mechanisms underlying these immunomodulatory effects remain elusive [7]. Several studies suggest that environmental, genetic, and epigenetic factors may contribute to RA. Epigenetic factors include microRNAs (miRNAs), which are small, non-coding RNAs that regulate gene expression via mRNA degradation or translational complex inhibition [8]. miRNAs play a crucial role in RA pathogenesis by influencing the immune response, inflammation, and tissue damage. Alterations in the expression profiles of miRNAs have been associated with increased secretion of proinflammatory cytokines, as well as with processes that cyclically maintain autoimmunity [9].

Recently, miRNAs has been highlighted as potential diagnostic and therapeutic biomarkers in RA, with their expression profiles demonstrating modifiability through alternative therapies such as acupuncture [10]. Acupuncture, a therapeutic method of traditional Chinese medicine, involves precise needle insertion into specific points of the body to elicit therapeutic effects. Characterized by low-cost and minimal adverse effects, this modality has shown efficacy in alleviating clinical symptoms of various diseases [11]. Accumulating evidence indicates that acupuncture can induce changes in the expression of miRNAs in multiple tissues and diseases, suggesting a molecular mechanism underlying these effects. Therefore, this study aims to identify RA-associated differentially expressed miRNAs, elucidate their mechanistic roles in disease pathogenesis, and evaluate their potential as biomarkers for monitoring acupuncture efficacy in RA management.

2 Materials and methods

2.1 miRNA selection

To evaluate the changes in miRNA expression profiles induced by acupuncture during RA pathogenesis, a search

was performed in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) of National Center for Biotechnology Information (NCBI). The search terms "rheumatoid arthritis" "microarray" "miRNA" and "expression" were used, which allowed us to identify only one study in a rat model of RA (accession number: GSE58460). miRNA expression data were obtained from the GEO dataset GSE58460, generated using the Agilent-031189 v16.0 microarray platform. The dataset includes samples from RA rat models before and after 34 d of acupuncture treatment. Raw data were downloaded in TXT format for subsequent analysis. This dataset was normalized using the robust multi-array analysis (RMA) method and analyzed to identify changes in expression profiles in the R v4.5.1 environment. Quality controls are shown in Supplementary Figure S1. Subsequently, a new search for miRNA expression data related to RA in humans was performed, where changes in miRNA expression profiles in the mononuclear cells of patients with RA were analyzed using the Affymetrix GeneChip miRNA 4.0 microarray technology (accession number: GSE124373). Five control microarray matrices and five RA case matrices were recovered. The files were retrieved in CEL format, normalized by the RMA method, and changes in miRNA expression profiles were analyzed through the R environment. For both data sets, a $|\log_2$ fold change (FC)| > 0.05 and $P < 0.05$ were taken as cutoff criteria [12]. Quality controls are shown in Supplementary Figure S2 and S3. Additionally, a literature search was conducted for miRNAs involved in RA pathogenesis and acupuncture intervention. The databases for miRNAs involved in RA were Medical Literature Analysis and Retrieval Systerm Online (MEDLINE), PubMed, American College of Physicians (ACP) Journal Club, Database of Abstracts of Reviews of Effectiveness (DARE), Scopus, Web of Science, and Ovid Excerpta Medica Database (EMBASE), covering the period from inception to July 1, 2025. A combination of controlled vocabulary and text words was used. The term "microRNA" was searched in MEDLINE, EMBASE, and others, and included more specific terms for individual miRNAs. For the broadest possible coverage, the search also included text words: mir, miRNA, microRNA. The same approach was used for RA: RA is used by MEDLINE, but EMBASE and others use arthritis, with more specific terms including cartilage or chondrocyte. Duplicates were removed, and the reference lists of all retrieved articles were reviewed without language restrictions to identify potentially relevant studies. For the candidate miRNAs, a Venn diagram was created considering the miRNAs shared among the three data sets.

2.2 Prediction of miRNA target genes

Target genes were selected using algorithms in the TargetScan (https://www.targetscan.org/vert_80/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/), miRmap (https://mirmap.ezlab.org/), and miRDB (https://mirdb.org/) databases. These algorithms identified nucleotide pairing in the 3' untranslated region (3'UTR) region of target mRNAs and the 5' seed region (2 – 7 nucleotides) of miRNAs. The target gene selection criterion was in at least three of the four databases [13].

2.3 Selection of differentially expressed genes

The target genes for candidate miRNAs were identified through comparative analysis between predicted targets and differentially expressed genes (DEGs) from microarray analysis. Genes shared between the two datasets were represented with a Venn diagram, ensuring the shared genes associated with RA and targets of RA-associated miRNAs.

2.4 Signaling pathway enrichment and functional network analysis

The genes shared between miRNA targets and DEGs were analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to identify biological processes, tissue expression enrichment, and human phenotype enrichment associated with RA, with a confidence score threshold of 0.4 (intermediate confidence). A network was created to represent the interaction between target genes and miRNAs using Cytoscape v3.7.2 software. The TSV file from STRING analysis was imported into the Cytoscape platform using a significance threshold of $P < 0.05$ or $q < 0.05$.

2.5 Drug interaction analysis

Genes involved in signaling pathways were analyzed in the Drug-Gene Interaction Database (DGIdb) v5.0 (https://dgidb.org/), which provides a search for drug targets by aggregating, categorizing, and curating drug and gene data. Subsequently, the identified drug-gene interactions were reviewed in the Kyoto Encyclopedia of Genes and Genomes (KEGG) DRUG database (https://www.genome.jp/kegg/drug/) to identify those drugs used in RA treatment. Finally, a literature review using the databases MEDLINE, PubMed, ACP Journal Club, DARE, Scopus, Web of Science, and Ovid EMBASE covering the period from inception to July 1, 2025, was performed for information on changes in the expression profiles of miRNAs induced by the drugs used in RA from the previous analysis. The search terms included: "expression" "miR-125a" "miR-125b" and "rheumatoid arthritis". The expression profiles of selected associated genes used in RA treatment are represented in a heatmap.

2.6 Drug-associated signaling pathway enrichment

The set of genes shared between miRNA targets and DEGs, which also interacted with various drugs, was

analyzed by ShinyGO v0.741 (http://bioinformatics.sdstate.edu/go74/) for enrichment analysis. The the KEGG pathways in *Homo sapiens* were applied to obtain the enrichment of signaling pathways associated with RA treatment. In contrast, STRING was applied to obtain the biological processes enrichment [Gene Ontology (GO)], tissue expression, and human phenotypes.

2.7 Statistical analysis

Differential expression analysis was performed using R v4.5.1 software, along with Bioconductor packages, for a comprehensive workflow. Quality control was assessed for each array to identify outliers. Systematic bias and technical variations between arrays were eliminated. During summarization, the intensities of multiple probes targeting the same gene were combined into a single expression value for each gene. For differential expression, linear models (lmFit in R) and empirical Bayes methods (eBayes) were used to evaluate differences in gene expression under experimental conditions.

3 Results

3.1 Selection of candidate miRNAs involved in RA

Differential expression analysis of human miRNAs showed 81 downregulated and 7 upregulated miRNAs on Affymetrix GeneChip miRNA 4.0 microarray (Figure 1A). In the murine model, 35 downregulated miRNAs and 15 upregulated miRNAs were observed on the Agilent-031189 microarray (Figure 1B) ($P < 0.05$). Using adjusted P values resulted in the considerable information loss, which may be attributable to extremely small effect sizes or high data variability. Based on the literature review of miRNAs implicated in RA, 179 miRNAs were obtained. Candidate miRNAs selection was performed based on a comparative analysis of the three datasets, ensuring that the selected miRNAs were implicated in RA with acupuncture treatment and showed an association with RA in human models (Figure 1C). The selected miRNAs were hsa-miR-125a-5p and hsa-miR-125b-5p.

3.2 Prediction of potential target genes for miRNAs

The potential target genes were predicted for the selected miRNAs based on their presence in at least three databases. For hsa-miR-125a-5p, 468 target genes were identified, and for hsa-miR-125b-5, 455 target genes were identified (Figure 1D). After removing duplicates, a total of 710 target genes from two datasets for both miRNAs were identified.

3.3 Expression microarrays and candidate genes selection

Differential expression analysis in the murine model showed 2 623 downregulated and 2 647 upregulated

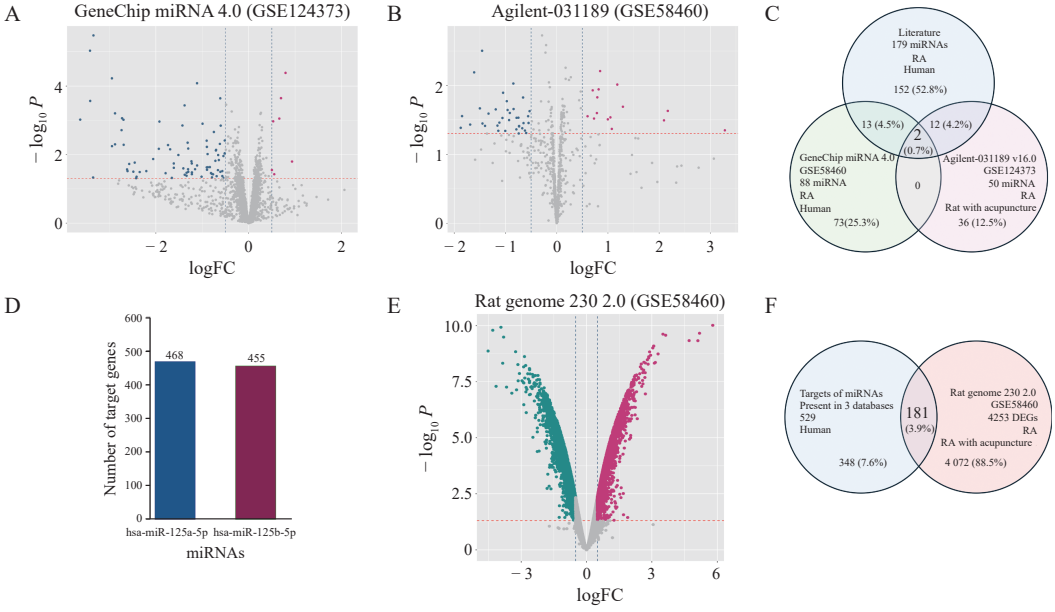

Figure 1 Selection of candidate miRNAs and their potential target genes

A, the volcano plot of differentially expressed miRNAs from human RA. B, the volcano plot of differentially expressed miRNAs from rat RA treated with acupuncture. Blue dots represent miRNAs with reduced expression (logFC < – 0.5, $P < 0.05$) and red dots represent miRNAs with increased expression (logFC > 0.5, $P < 0.05$). C, Venn diagram between human and rat miRNAs and systematic literature review. D, target genes of the selected miRNAs. E, DEGs in RA rats treated with acupuncture. Green dots represent genes with reduced expression (logFC < – 0.5, $P < 0.05$) and pink dots represent genes with increased expression (logFC > 0.5, $P < 0.05$). F, Venn diagram between miRNA target genes and DEGs in rats.

genes (Figure 1E) on the Affymetrix Rat Genome 230 2.0 expression microarray. Comparative analysis of miRNA target genes and Rat Genome 230 2.0 microarray-derived DEGs revealed 181 genes commonly identified as miRNA targets, acupuncture-responsive, and conserved in humans (Figure 1F).

3.4 Pathway and biological process analysis

The genes in the comparative analysis were subjected to GO enrichment, tissue expression enrichment, and human phenotype enrichment using STRING. Biological process enrichment identified the extrinsic apoptotic signaling pathway, protein localization in vacuoles, establishment of protein localization in vacuoles, and negative regulation of cellular metabolic processes (Figure 2A). For tissue expression enrichment, the following pathways were enriched for tissues, cell types, enzyme sources, animal tissues, and endocrine glands (Figure 2B). For human phenotype enrichment, the most enriched pathways were reticulocyte measurement, non-albumin serum protein measurement, and hematological measurement (Figure 2C).

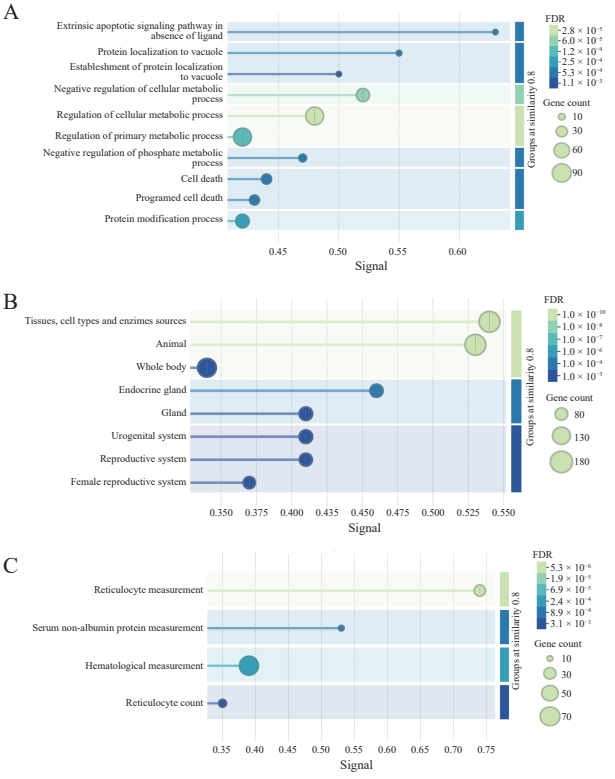

Figure 2 Biological processes associated with RA pathogenesis

A, GO biological process enrichment. B, tissue expression enrichment. C, human phenotype (Monarch database) enrichment. The size of the circles indicates the number of genes involved in each process, and the color bar indicates the false discovery rate (FDR). Red nodes represent miRNAs, blue nodes represent genes, and gray lines represent interactions.

3.5 Construction of interaction network

To explore the functional associations between the selected miRNAs and their potential target genes, the list of 181 genes involved in signaling pathways was analyzed using Cytoscape v3.10.2 to generate an interaction network between the selected miRNAs and their potential target genes. The results showed the formation of 309 edges, an average node degree of 3.32, an average local clustering coefficient of 0.455, an expected number of edges of 166, and a gene-gene interaction enrichment value of 1.0×10^{-16} (Figure 3).

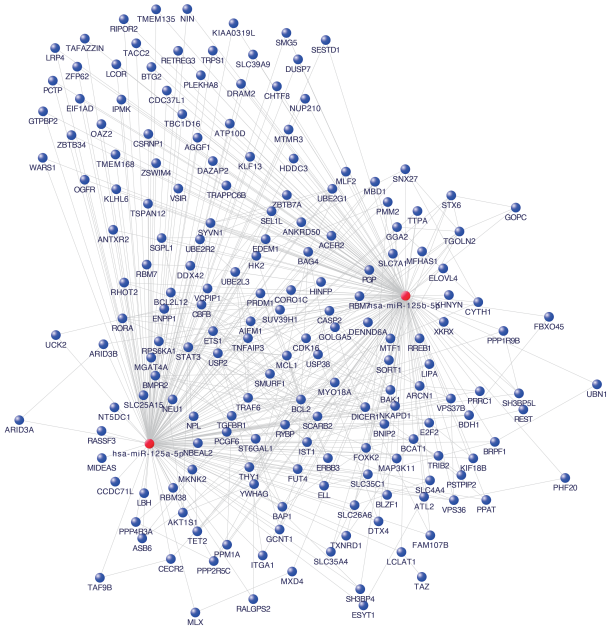

Figure 3 Interaction network between hsa-miR-125a-5p/hsa-miR-125b-5p and their predicted target genes

Red nodes represent miRNAs, blue nodes represent genes, and gray lines represent interactions. The thickness of the line indicates the strength of the data support.

3.6 Gene-drug interactions implicated in RA

A total of 135 drugs were subjected to enrichment analysis in the KEGG DRUG database, which identified 58 drugs in RA treatment that interacted with 22 genes involved in this disease, targeting the biomarkers of hsa-miR-125a-5p and hsa-miR-125b-5p (Table 1). The expression profiles of these genes exhibited changes following acupuncture treatment (Figure 4A), targeting rno-miR-125a-5p and rno-miR-125b-5p, whose expression decreased in RA and maintained its normal level after acupuncture treatment in the murine model (Figure 4B). These data were consistent with the hsa-miR-125a-5p and hsa-miR-125b-5p expression levels in normal human subjects, where the expression levels of these

Table 1 Gene-drug interactions and their association with RA

Gene symbol	Drug	Indication	Effect on RA	Interaction score
TET2	Rituximab	Antineoplastic agent	Anti-rheumatic	0.483 3
	Selinexor	Not mentioned	Anti-inflammatory	1.740 1
	Olaparib	Not mentioned	Anti-inflammatory	0.189 1
	Dasatinib anhydrous	Antineoplastic agent	Kinase inhibition	0.114 4
	Hydroxyurea	Antineoplastic Agents	Anti-inflammatory	0.414 3
PPM1A	Nortriptyline	Antiasthmatic	Analgesic	3.262 7
TXNRD1	Arsenic trioxide	Antineoplastic	Effect on microbiota	1.003 9
	Ascorbic acid	Not mentioned	Anti-inflammatory	0.669 2
	Aurothioglucose	Not mentioned	Anti-inflammatory	8.700 6
RIPOR2	Rifampin	Not mentioned	Anti-rheumatic	5.593 2
BAP1	Everolimus	Immunosuppressant	Analgesic	0.191 9
	Olaparib	Not mentioned	Anti-inflammatory	0.141 8
	Vorinostat	Antineoplastic agent	MMPs inhibitor	0.116 5
	Sunitinib	Antineoplastic agent	Anti-angiogenetic	0.135 9
MKNK2	Sorafenib	Antineoplastic agent	Anti-inflammatory	0.021 6
	Gefitinib	Antineoplastic agent	Anti-inflammatory	0.037 1
	Erlotinib	Antineoplastic agent	Anti-inflammatory	0.044 3
MGAT4A	Cetuximab	Antineoplastic agent	Anti-inflammatory	0.705 4
BCL2	Oxaliplatin	Not mentioned	Pro-inflammatory	0.050 2
	Methylprednisolone	Anti-Inflammatory	Anti-inflammatory	0.106 1
	Paclitaxel	Peripheral arterial	Anti-inflammatory	0.023 1
	Bortezomib	Antineoplastic agent	Anti-inflammatory	0.0074
	Erythromycin	Not mentioned	Anti-inflammatory	0.055 3
	Ribavirin	Not mentioned	Anti-inflammatory	0.083 7
	Pentoxifylline	Lateral sclerosis (ALS)	Anti-inflammatory	0.036 3
	Hydroquinone	Not mentioned	Anti-inflammatory	0.074 8
	Cisplatin	Not mentioned	Anti-inflammatory	0.017 3
	Selenium	Not mentioned	Anti-inflammatory	0.112 3
	Docetaxel anhydrous	Antineoplastic agent	Chemotherapy	0.007 6
	Edaravone	Not mentioned	Inhibition C-reactive protein	0.127 3
	Ursodiol	Polorectal polyps	Anti-inflammatory	0.079 5
YWHAG	Daunorubicin hydrochloride	Not mentioned	Anti-inflammatory	0.790 9
ERBB3	Gefitinib	Antineoplastic agent	Chemotherapy	0.044 9
	Cetuximab	Antineoplastic agent	Chemotherapy	0.027 1
	Selumetinib	Not mentioned	Anti-inflammatory	0.030 4
	Vandetanib	Antineoplastic agent	Chemotherapy	0.017 3
	Curcumin	Not mentioned	Anti-inflammatory	0.030 4
	Momelotinib	Not mentioned	Anti-inflammatory	0.077 2
	Erlotinib	Antineoplastic agent	Anti-inflammatory	0.017 9
PRDM1	Simvastatin	Antidyslipidaemic	Anti-inflammatory	0.029 8
	Atorvastatin calcium	Anticholesterolaemic	Increased arterial stiffness	0.027 1
	Rosuvastatin	Antihypecholesterolemic	Anti-inflammatory	0.062 1
	Fluvastatin	Antihypecholesterolemic	Bone resorption regulator	0.149 1
STAT3	Digitoxin	Anti-arrhythmia agents	Inflammation/angiogenesis	0.091 5
	Celecoxib	NSAID	Analgesic	0.051 9
	Digoxin	Anti-arrhythmia agents	Anti-rheumatic	0.051 1
	Niclosamide	Not mentioned	Induces apoptosis	0.064 4

Table 1 Continued

Gene symbol	Drug	Indication	Effect on RA	Interaction score
STAT3	Pyrimethamine	Not mentioned	Reduce edema	0.316 3
LIPA	Trifluoperazine	Antipsychotic	Inflammatory response	0.580 0
PGP	Paclitaxel	Peripheral arterial	Anti-inflammatory	0.158 1
RORA	Interferon beta-1a	Antineoplastic	Anti-inflammatory	1.740 1
	Citalopram	Antidepressant	Anti-inflammatory	0.745 7
	Melatonin	Hypnotics and sedatives	Hypnotics and sedatives	1.350 0
PPAT	Azathioprine	Not mentioned	Analgesic	0.915 8
	Azathioprine sodium	Not mentioned	Analgesic	17.40 1
	Mercaptopurine	Antineoplastic agents	Analgesic	0.773 3
TNFAIP3	Methotrexate	DMARD	inhibits the synthesis DNA	0.949 1
BMPR2	Dextromethorphan polistirex	Antitussive agents	Anti-inflammatory	0.543 7
ST6GAL1	Floxacillin	Not mentioned	inhibits the synthesis DNA	11.600 0
USP2	Tannic acid	Not mentioned	Anti-inflammatory	0.200 7
	Hexachlorophene	Not mentioned	Anti-inflammatory	0.048 9
	Curcumin	Not mentioned	Inhibition C-reactive protein	0.030 4
AIFM1	Cyclosporine	Immunosuppressant	Immunosuppressant	0.600 0
CBFB	Daunorubicin liposomal	Antineoplastic	Anti-inflammatory	0.017 9

TET2, ten-eleven translocation 2. *PPM1A*, protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1A. *TXNRD1*, thioredoxin reductase 1. *RIPOR2*, Rho family interacting cell polarization regulator 2. *BAP1*, BRCA1 associated protein 1. *MKNK2*, MAP kinase interacting serine/threonine kinase 2. *MGAT4A*, mannosyl (α-1,3-)-glycoprotein β-1,4-N-acetylglucosaminyltransferase, isozyme A. *BCL2*, B-cell lymphoma 2. *YWHAG*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma. *ERBB3*, Erb-B2 receptor tyrosine kinase 3. *PRDM1*, BLIMP-1-B lymphocyte-induced maturation protein 1. *STAT3*, signal transducer and activator of transcription 3. *LIPA*, lipase A, lysosomal acid type. *PGP*, phosphoglycolate phosphatase. *RORA*, RAR related orphan receptor A. *PPAT*, phosphoribosyl pyrophosphate amidotransferase. *TNFAIP3*, tumor necrosis factor alpha-induced protein 3. *BMPR2*, bone morphogenetic protein receptor type 2. *ST6GAL1*, ST6 beta-galactoside alpha-2,6-sialyltransferase 1. *USP2*, ubiquitin specific peptidase 2. *AIFM1*, apoptosis-inducing factor, mitochondrion-associated 1. *CBFB*, core-binding factor subunit beta.

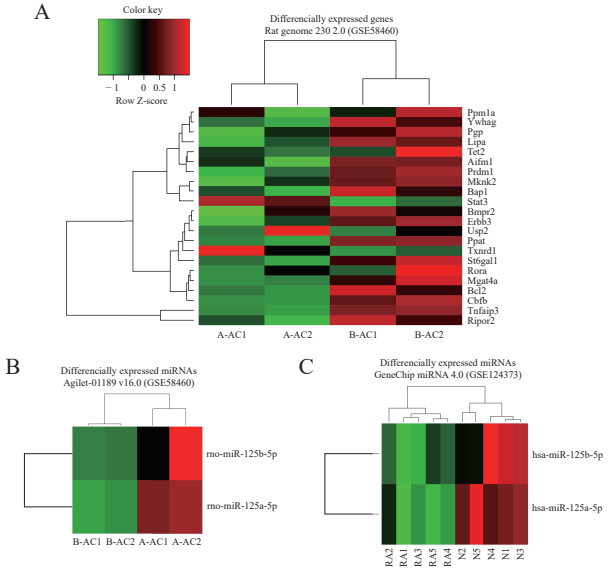

Figure 4 DEGS and miRNAs involved in RA

A, heatmap of differentially expressed miRNA target genes following acupuncture stimulation. B, heatmap of changes in rat miRNA expression before and after acupuncture stimulation. C, heatmap of changes in human miRNA expression between standard control and RA groups. A-AC: after acupuncture. B-AC: before acupuncture. N: normal.

miRNAs elevated in healthy individuals and decreased in RA patients (Figure 4C). Therefore, these miRNAs could play a role as biomarkers for monitoring acupuncture treatment in RA.

The role of 58 drugs on the expression profiles of hsa-miR-125a-5p and hsa-miR-125b-5p in various diseases, including RA, was explored hypothetically (Table 2, Figure 5) [14–26].

The potential binding sites of target genes were analyzed via the seed region of hsa-miR-125a-5p (Supplementary Table S1) and hsa-miR-125b-5p (Supplementary Table S2) with their respective 3'UTR regions of the mRNAs, as well as their percentile scores. Finally, the signaling pathway enrichment analysis was performed using ShinyGO v0.741, intergrating drug-intervention data with the 22 target genes of hsa-miR-125a-5p and hsa-miR-125b-5p. The results revealed 20 significantly enriched pathways involved in metabolic regulation, immunological responses, necroptosis, and cellular differentiation processes (Figure 6).

Table 2 Drugs-induced overexpression of hsa-miR-125 family members in different diseases

Drug	miRNA	Model	Expression level	Reference
Rituximab	miR-125b	Human	Overexpression of miR-125b in RA is associated with a good response to treatment	[14]
Olaparib	miR-125a-3p	Human	Olaparib induces miR-125a-3p expression in patients with ovarian cancer	[15]
Hydroxyurea	miR-125a-5p	Human	Hydroxyurea induces miR-125a-5p expression in sickle cell disease	[16]
Arsenic trioxide	miR-125b-5p	Mouse	Resistance to doxorubicin and arsenic trioxide in acute promyelocytic leukemia has been found to be associated with miR-125b-5p	[17]
Ascorbic acid	miR-125b-5p	Human mesenchymal stem cells	Ascorbic acid and dexamethasone are associated with increased miR-125b-5p expression during cell differentiation	[18]
Sorafenib	miR-125b-5p	HCC, PLC/PRF5, and Hep3B cell lines	Sorafenib induces miR-125b-5p expression in renal carcinoma cells	[19]
Gefitinib	miR-125a-5p	HNE-1 and HK-1 cell lines	Gefitinib induces miR-125a-5p expression in nasopharyngeal carcinoma cell lines	[20]
Erlotinib	miRNA-125a-5p	A549 cell line	Erlotinib induces miR-125a-5p expression in lung cancer	[21]
Cetuximab	miR-125b-5p	Human	Cetuximab is associated with increased miR-125 expression in colorectal cancer cells	[22]
Methylprednisolone	miR-125b-5p	Human	Methylprednisolone is associated with increased miR-125b-5p expression in pancreatic ductal adenocarcinoma	[23]
Paclitaxel	miR-125a-5p	Human	Up-regulation of miR-125a-5p inhibited paclitaxel resistance in Endometrial Carcinoma	[24]
Bortezomib	miR-125b-5p	Human	Bortezomib repressed cMyc and simultaneously induced miR-125b-5p that exerted a cytoprotective effect through the downmodulation of MAD4 in T-cell lymphoma	[25]
Cisplatin	miR-125b-5p	Human	miR-125b-5p up-regulation promoted cell death in gallbladder cancer cells in the presence of cisplatin	[26]

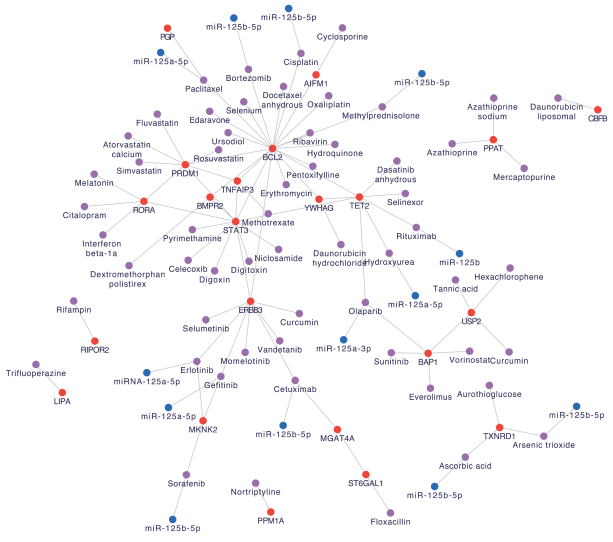

Figure 5 Gene-drug-miRNA interaction network

Twenty-two genes (red) are showing interaction with 58 drugs (purple), which could modulate changes in the expression profiles of miR-125 family members (blue) in various diseases, including RA.

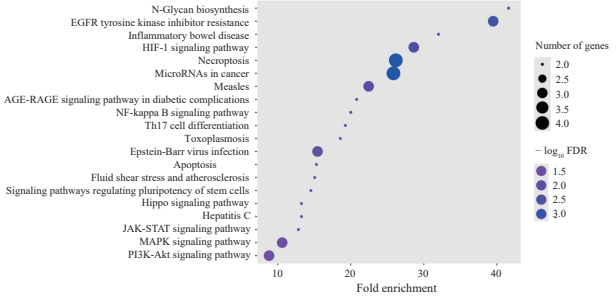

Figure 6 Pathway enrichment of RA-associated targets of hsa-miR-125a-5p/hsa-miR-125b-5p with drug interactions

The size of the circles indicates the genes numbers involved in each process, and the color bar indicates the FDR.

4 Discussion

The present study investigated the potential of miRNAs as biomarkers for monitoring acupuncture efficacy in RA management. It has recently been reported that different

types of molecules respond to acupuncture treatment in various diseases, for example, in cancer survivors who with insomnia, the genes catechol-O-methyltransferase (*COMT*) and nuclear factor kappa B subunit 2 (*NFKB2*) are potentially associated with the response to acupuncture [27], while in murine models with hypertension, acupuncture was observed to induce changes in expression at the protein level, modifying the proteins synapsin-1, pyruvate kinase isoenzyme (SYN1), NAD-dependent deacetylase sirtuin (SIRT), inhibitor of kappa B alpha (IκBα), ubiquitin hydrolase isoenzyme L1 (UCHL1), and myelin basic protein (MBP) [28]. At the transcriptomic level, it has been observed that electroacupuncture at Neiguan (PC6) inhibits myocardial fibrosis by regulating the expression of long non-coding RNAs (lncRNA), which could be considered as a potential therapeutic method for the treatment of this disease [29]. Finally, it has been reported that some miRNAs respond to acupuncture in various diseases, with dysregulated miRNAs exhibiting notable changes in their expression profiles following therapeutic interventions [10]. Recently, existing studies indicated the capacity of acupuncture to restore disease-specific miRNAs involved in dysfunctions and physical abnormalities. However, it is essential to highlight that these types of studies present variations in their hypotheses and methodologies [30]. The advancements in standardized algorithms have also enabled massive information processing analysis, enabling biomarker discovery of new drugs, diagnostic strategies, and therapeutic monitoring across various diseases [31-35].

Our analysis establishes that reduced expression levels of hsa-miR-125a-5p and hsa-miR-125b-5p are associated with RA pathogenesis and progression, meanwhile these results are consistent with studies from murine models and clinical cohorts, where diminished serum levels of these miRNAs are linked to RA onset [36], while increased expression is associated with favorable therapeutic responses to drug treatment [14]. Both hsa-miR-125a-5p and hsa-miR-125b-5p target genes regulate critical pathways implicated in RA progression, suggesting their modulation of these miRNAs may serve as a therapeutic strategy to improve disease management.

Biological process analysis reveals that hsa-miR-125a-5p and hsa-miR-125b-5p target genes are involved in the extrinsic apoptotic signaling pathway under ligand-independent condition during RA pathogenesis. This dysregulation promotes apoptosis through mechanisms, including the release of granzyme B and perforin by lymphocytes, or by activation of the complement pathway, leading to hypercitrullination in response to cellular stress and immune-mediated mechanisms in RA [37]. The autophagy-related "protein localization to vacuole" mechanisms also contribute to RA progression by facilitating the accumulation of misfolded proteins, leading to joint damage and inflammation [38]. Other biological processes, such as "programmed cell death" encompass both apoptosis and autophagy, which play a critical role in RA progression [39]. Enrichment analysis of human tissue-specific expression and phenotypes, such as "endocrine gland" reveal endocrine dysfunction (e.g., hypothyroidism, adrenal insufficiency, and sex hormone imbalances), as a potential contributor to RA pathogenesis, driven by hormonal receptor expression in synovial tissues [40]. These results demonstrate the pivotal role of miRNA-targeted genes in RA development and progression.

Our analysis demonstrated that basal expression levels of rno-miR-125a-5p and rno-miR-125b-5p are downregulated in the rat model of RA, while acupuncture treatment induced significant upregulation of these miRNAs. This pattern aligns with observations in pharmacological treatments targeting RA: rituximab therapy administrated over three months is associated with elevated serum levels of hsa-miR-125b-5p in RA patients and B-cell lymphomas [11], and Sorafenib, as a multikinase inhibitor, suppressing cell proliferation and angiogenesis, elicited comparable miRNA modulation in preclinical models [41]. Erlotinib is a tyrosine kinase inhibitor (TKI) used in lung cancer treatment. However, most patients may develop resistance to this agent. Therefore, these findings suggest that hsa-miR-125a-5p could act as a tumor suppressor at the therapeutic levels, since high levels of expression of this miRNA are associated with a decrease in cellular protein, apoptosis and sensitivity of lung cancer cells [23]. We also hypothesized that restoring the expression levels of hsa-miR-125a-5p and hsa-miR-125b-5p could play a key role as monitoring molecules for pharmacological and non-pharmacological treatment, such as acupuncture intervention [42, 43]. Although evidence regarding the hsa-miR-125a-5p and hsa-miR-125b-5p as biomarkers for treatment follow-up and monitoring in various diseases are consistent across studies, our proposal to use them as biomarkers for monitoring acupuncture treatment should be viewed with caution, as the application of bioinformatics tools alone may be affected by bias, data errors, and limitations of the applied algorithms. Therefore, it is essential to confirm these findings through experiments using biological samples to ensure their biological relevance and reliability.

Acupuncture, as a therapeutic intervention in RA, seeks to correct disturbances between self-controlled systems by analyzing all symptoms and signs, which allows the activation of the system's connection, and improves human resistance [44]. This approach demonstrates potential benefits for symptomatic treatment of RA. Two trials evaluated the intervention of traditional acupuncture and analgesia. Trial A was conducted on RA patients aged over 18 years, who underwent acupuncture over a period of ten weeks, with twenty sessions for 30 min, stimulating six main points: Quchi (LI11), Waiguan (TE5), Zusanli

(ST36), Yanglingquan (GB34), Waiqiu (GB36), and Xuanzhong (GB39). Trial B involved RA patients who received ten acupuncture sessions over five weeks, with a needle retention time of 20 min, stimulating 16 points: Sishencong (EX1), Xiyan (EX27), Qihai (CV6), Zhongwan (CV12), Hegu (LI4), Mingmen (GV4), Dazhui (GV14), Taichong (LR3), Neiguan (PC6), Sanyinjiao (SP6), Zusanli (ST36), Dashu (BL11), Pishu (BL20), Sanjiaoshu (BL22), Shenshu (BL23), and Kunlun (BL60). Both studies showed favorable results in pain reduction [45, 46]. However, the sex of the patients, age, time, and stimulation points may introduce variations in treatment outcomes. Therefore, monitoring RA treatment could significantly improve patient health. However, these variables may modify miRNA expression profiles (e.g., hsa-miR-125a-5p and hsa-miR-125b-5p), underscoring the need for patient-specific validation to ensure therapeutic relevance.

The miR-125 family has been implicated in regulating signaling pathways involved in RA pathogenesis. The activity of the PI3K/Akt/mTOR signaling pathway is involved in multiple pathologies including tumors and inflammatory diseases, where the inactivation of this pathway can affect renal epithelial tubular inflammation [47]. miR-125 can regulate poly(ADP-ribose) polymerase 2 (PARP2) via the PI3K/Akt/mTOR pathway, and its dysregulation is associated with severe hyperplasia and infiltration of inflammatory cells in murine models. The reconstitution of miR-125 expression can attenuate RA progression by inhibiting PARP2 expression [48].

Functional analysis of hsa-miR-125a-5p and hsa-miR-125b-5p reveals their shared regulatory capacity over multiple target genes, as determined by their complementarity in the seed region [49]. Network analysis demonstrates that each target gene can be regulated by multiple miRNA family members. Recent studies have reported that these miRNAs and their respective target genes are involved in RA pathogenesis via critical signaling pathways. For example, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway plays a central role in joint inflammation and destruction. Activation of this pathway promotes the production of proinflammatory cytokines (e.g., TNF-α and IL-1β), and contributes to the proliferation of fibroblast-like synoviocytes (FLS), which are responsible for synovial hyperplasia causing cartilage damage [50]. JAK-STAT signaling pathway is an intracellular pathway activated by cytokines, which can cause inflammation and tissue damage through aberrant immune responses [51].

The MAPK signaling pathway plays a crucial role in RA pathogenesis by regulating inflammatory processes and joint destruction [52]. The PI3K-Akt signaling pathway is implicated in the abnormal proliferation of fibroblast-like synoviocytes, synovial inflammation, and inflammatory cytokine production, with its dysregulation emerging as a promising therapeutic approach for RA management [53]. Notably, TNF-α pathway is also regulated by miRNAs, such as miR-19 and miR-20, which have an anti-inflammatory function and modify toll-like receptor (TLR) and MAPK [54].

This study presents methodological strengths and weaknesses. The strengths include systematic normalization protocol, differential expression of miRNAs, genes from microarray data, and the search for potential target genes for each miRNA, which allowed these results to be replicated. The selection of miRNA candidates integrated cross-species data from human RA cohorts and murine models under diverse experimental conditions, ensuring disease-specific relevance and treatment-specific differential expression patterns. Key limitations include restricted generalizability due to uncontrolled confounding variables, such as the effect of diet, lifestyle, smoking, alcohol consumption, temperature, presence of migraines, ethnic origin, among others. In the murine model, however, we do not control for variables such as diet, circadian rhythm, male or female selection, and other variables that can have a confounding effect. While mechanistic analyses identified putative miRNA targets, their pleiotropic roles in RA pathogenesis remain incompletely characterized. These findings require independent validation through targeted experiments using RA patient-derived biological specimens. Our findings implicated miRNAs as key regulators in RA pathogenesis and may respond to the acupuncture-induced therapeutic effects. Furthermore, acupuncture applied to specific points may induce selective changes in miRNA expression.

5 Conclusion

The miR-125 family (hsa-miR-125a-5p and hsa-miR-125b-5p) has been implicated in RA pathogenesis through dysregulated expression patterns, while reduced expression levels correlate with disease progression. The reconstitution of the expression levels of these miRNAs has been associated with an improvement in RA patients, establishing their potential as diagnostic and prognostic biomarkers. In this *in silico* study, our results suggest that acupuncture can induce the reconstitution of hsa-miR-125a-5p and hsa-miR-125b-5p expression profiles, indicating their potential as biomarkers for therapeutic response monitoring in RA management.

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Competing interests

The authors declare no conflict of interest.

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hsa-miR-125a-5p 与 hsa-miR-125b-5p 作为监测针灸治疗类风湿性关节炎潜在生物标志物的计算机评估研究

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【摘要】目的 基于生物信息学方法筛选与类风湿性关节炎（RA）发病机制相关的差异表达 microRNA（miRNA），并评估其作为针灸疗效及监测生物标志物的潜力。**方法** 从人类 RA 组织及针灸干预的 RA 小鼠模型中获取 miRNA 微阵列数据（CEL 与 TXT 格式）。采用稳健多阵列平均（RMA）算法进行数据标准化与差异表达分析。通过比较人类 RA 组织、小鼠 RA 模型（经针灸处理）以及文献中与 RA 发病及针灸治疗相关的差异表达 miRNA，鉴别出候选 miRNA。利用生物信息学工具预测每个 miRNA 的潜在靶基因，并通过相互作用基因/蛋白质检索工具（STRING）与 ShinyGO 工具进行通路富集分析。基于药物-基因相互作用数据库（DGBIdb）数据集进行药物-基因相互作用分析。使用 Cytoscape v3.10.3 构建基因相互作用网络。**结果** 分析结果鉴定出 hsa-miR-125a-5p 与 hsa-miR-125b-5p 作为与 RA 发病机制相关的潜在生物标志物，分别对应 468 和 455 个靶基因。这些靶基因显著富集于 20 条信号通路，包括 Janus 激酶-信号转导子及转录激活子（JAK-STAT）信号通路、丝裂原活化蛋白激酶（MAPK）信号通路、磷脂酰肌醇 3-激酶-蛋白激酶 B（PI3K-Akt）信号通路及核因子（NF）-κB 信号通路，这些通路均与 RA 的发生和进展相关。药物-基因相互作用网络显示，有 22 个基因与 58 种 RA 治疗药物存在显著关联，其中 13 个基因与 hsa-miR-125 家族成员存在相互作用。**结论** hsa-miR-125a-5p 与 hsa-miR-125b-5p 可能通过调控 JAK-STAT、MAPK、PI3K-Akt 和 NF-κB 信号通路，在 RA 发病机制中具有重要的调控作用。本研究提示 hsa-miR-125a-5p 与 hsa-miR-125b-5p 在包括 RA 的多种疾病药物干预下呈现差异表达，提示其有作为监测针灸治疗的潜在生物标志物的潜力。尽管现有证据表明针灸可改变 miRNA 表达谱，但仍需通过生物学模型进一步验证。

【关键词】针灸；类风湿性关节炎；软骨；软骨细胞；生物标志物；生物信息学；miRNA