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MITOCHONDRIAL DYSFUNCTION ACROSS SYNOVIAL AND PERIPHERAL COMPARTMENTS IN RHEUMATOID ARTHRITIS: A MULTI-TISSUE TRANSCRIPTOMIC INTEGRATION OF PBMC, SYNOVIAL BIOPSY AND WHOLE BLOOD

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Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune disease with synovial inflammation and systemic immune alterations. Mitochondrial dysfunction has been implicated in RA pathogenesis, but its tissue-specific and shared molecular signatures remain poorly characterized. We performed an integrative transcriptomic analysis of three public datasets: GSE17755 (PBMC), GSE89408 (synovial biopsy), and GSE93272 (whole blood). Differentially expressed genes (DEGs) were identified ($P<0.05$, $|log2FC| \geq 1$) and intersected with MitoCarta 3.0 to obtain mitochondria-related DEGs (mito-DEGs). Protein-protein interaction networks, hub genes (identified using the MCC algorithm), functional enrichment (GO/KEGG), and regulatory networks (miRNA-TF-mRNA) were constructed. PBMC, synovial biopsy, and whole blood yielded 37, 220, and 119 mito-DEGs, respectively. Only one gene (*AKAP1*) was common across all three tissues. Hub genes predominantly encoded subunits of mitochondrial respiratory chain complexes (NDUF family and UQCR genes). Enriched pathways included oxidative phosphorylation, thermogenesis, and neurodegeneration-related pathways. Regulatory analysis identified FOXC1, GATA2, and hsa-miR-16-5p as key transcriptional and post-transcriptional regulators. Mitochondrial dysfunction in RA is tissue-specific, but converges on the dysregulation of oxidative phosphorylation. *AKAP1* emerges as a potential systemic mitochondrial biomarker, pending validation in independent cohorts. These findings provide a multi-tissue resource for targeting mitochondrial pathways in RA.

Keywords: Rheumatoid arthritis, Mitochondrial dysfunction, Transcriptomics, Oxidative phosphorylation, Biomarker

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1. Introduction

Rheumatoid arthritis (RA) is a long-term systemic autoimmune condition that involves sustained synovial inflammation, advancing joint erosion, and numerous non-articular symptoms (Smolen et al., 2016). The condition affects nearly 0.5–1% of the world's population and constitutes a primary cause of disability as well as lower quality of life (Finckh et al., 2022; Uke et al., 2025). Though the definitive etiology of RA remains undetermined, it is widely recognized that a multifaceted interaction among genetic susceptibility, environmental agents, and immune dysregulation underlies both the initiation and progression of the disease (Alivernini et al., 2022).

A growing collection of recent evidence has revealed that cellular metabolism is a key regulator of both immune responses and inflammatory mechanisms (Rahmati et al., 2024). Among metabolic organelles, mitochondria have emerged as key regulators of immune cell activation, oxidative stress, and inflammatory signaling pathways (Gao et al., 2024). Beyond their classical function in ATP production, mitochondria participate in reactive oxygen species (ROS) generation, apoptosis regulation, and innate immune signaling (West and Shadel, 2017).

Several autoimmune and inflammatory disorders, including RA, have been associated with abnormalities in mitochondrial function (Huang et al., 2026). In RA specifically, altered mitochondrial dynamics, oxidative phosphorylation imbalance, increased ROS production, and mitochondrial DNA damage have all been reported in both synovial tissue and circulating immune cells (Fearon et al., 2016; Ma et al., 2022). A recent study demonstrated that peripheral blood mitochondrial DNA gene variation and methylation levels are significantly associated with RA pathogenesis, highlighting the systemic nature of mitochondrial dysfunction in this disease (Huang et al., 2026). Furthermore, mitochondrial dysfunction in RA has been linked to the differentiation and activation of pro-inflammatory T cell subsets, particularly T_H17 cells, which play a central role in disease pathogenesis (Weyand and Goronzy, 2017).

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The pathogenesis of RA involves multiple tissues and cellular compartments, including synovial tissue, circulating immune cells (PBMCs), and peripheral blood components (Smolen et al., 2018). However, most transcriptomic studies to date have profiled only a single tissue type at a time, which limits the ability to distinguish molecular changes that are truly systemic from those confined to the local inflammatory site (Smiljanovic et al., 2020; Binvignat et al., 2024; Bellocchi et al., 2025). This is also true of studies specifically addressing mitochondrial gene signatures in RA: for example, a 2025 study investigating mitochondrial apoptosis-related genes (MARGs) identified hub genes such as *MRPS10* and *TUFM* and demonstrated their diagnostic potential through ROC analysis, with transcription factors such as YY1 and FOXO3 emerging as regulators but did so within a single tissue compartment (Gao et al., 2025). Similarly, work on the MTHFD2-CKMT1 axis established a role for oxidative phosphorylation in RA-associated osteoclastogenesis and bone loss, again within a single experimental system (Li et al., 2025). Because mitochondrial alterations in these earlier reports were characterized in isolation, it remains unclear which mitochondrial changes are shared across the synovial, PBMC, and whole-blood compartments (and are therefore more likely to reflect systemic RA pathophysiology and represent viable blood-based biomarkers), and which are tissue-restricted responses to the local inflammatory microenvironment of the joint. Integrative, multi-tissue analysis is needed to resolve this distinction and to provide a more comprehensive picture of the disease-associated molecular networks underlying RA (Tariq et al., 2025). To date, however, no study has systematically compared mitochondrial dysfunction across synovial, PBMC, and whole blood compartments within a single integrative framework, leaving the shared and tissue-specific mitochondrial architecture of RA undefined. Critically, this gap concerns the cross-compartment comparability of mitochondrial changes — that is, which alterations are shared versus tissue-restricted — rather than whether mitochondrial dysfunction occurs in RA at all, a point already well established in the literature.

Here, we resolve this distinction by conducting an integrative transcriptomic analysis of publicly available gene expression datasets derived from PBMCs, synovial biopsy tissue, and whole blood from patients with RA. We identified differentially expressed genes and then used the MitoCarta 3.0 database to pinpoint those related to mitochondrial function. Subsequent analyses, including functional enrichment, protein–protein interaction network analysis, and miRNA–TF–mRNA regulatory network analysis, were performed to interpret the biological significance and regulatory landscape of mitochondrial gene signatures across tissues. By adopting this multi-tissue strategy, we aim to elucidate the role of mitochondrial dysfunction in RA and to identify potential mitochondrial biomarkers that may improve our understanding of disease mechanisms and inform future treatment strategies.

2. Materials and Methods

2.1. Data Acquisition from GEO Database

Publicly available gene expression datasets relevant to RA were downloaded from the Gene Expression Omnibus (GEO) repository. Three independent transcriptomic datasets representing distinct biological compartments involved in RA pathogenesis were included in this study: GSE17755 (peripheral blood mononuclear cells, PBMC), GSE89408 (synovial biopsy tissue), and GSE93272 (whole blood).

We analyzed datasets generated by three different expression-profiling platforms. GSE17755 was processed on GPL1291 (Hitachisoft AceGene Human Oligo Chip 30K, two-color microarray), GSE93272 on GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array, microarray), and GSE89408 on GPL11154 (Illumina HiSeq 2000, RNA sequencing), respectively. The PBMC dataset (GSE17755) consisted of 112 RA cases and 45 healthy individuals. The synovial biopsy dataset (GSE89408) included 152 RA samples and 28 healthy-control samples. The whole blood dataset (GSE93272) included 245 RA samples and 30 healthy controls. Expression profiling was performed by microarrays for GSE17755 and GSE93272 and by RNA sequencing for GSE89408. A summary of dataset characteristics is provided in Table 1.

Table 1. Summary of GEO dataset characteristics

GEO Accession	GEO Platform (GPL)	Control (n)	Case (n)	Sample Type	Experimental Design
GSE17755	GPL1291	45	112	PBMC	Expression profiling by array
GSE89408	GPL11154	28	152	Synovial biopsy	Expression profiling by throughput sequencing
GSE93272	GPL570	30	245	Whole blood	Expression profiling by array

GEO= gene expression omnibus, PBMC= peripheral blood mononuclear cells

2.2. Differential Gene Expression Analysis

Differential gene expression analysis was carried out independently for each dataset using the GEO2R web-based platform (https://www.ncbi.nlm.nih.gov/geo/geo2r/; accessed April/2026). GEO2R performs differential expression testing directly on the processed, submitter-normalized expression matrices deposited in GEO; no additional re-normalization was performed. For the two microarray datasets, probe-to-gene mapping was based on the platform-specific annotation files (GPL1291 for GSE17755; GPL570 for GSE93272), which GEO2R automatically applies to assign gene symbols to each probe or probeset. For the RNA-seq dataset (GSE89408, GPL11154), expression values were provided as gene-level RSEM counts, so a probe-to-gene mapping step was unnecessary; GEO2R applied its RNA-seq differential expression workflow (voom-transformed counts with limma) to this dataset rather than the array-based linear model used for the two microarray datasets.

For each dataset (GSE17755, GSE89408, and GSE93272), samples were divided into two groups: RA patients and healthy controls.

The Benjamini–Hochberg false discovery rate (FDR) correction method was employed to calculate adjusted p-values, thereby controlling for multiple hypothesis testing when determining statistical significance. Only genes with an adjusted p-value < 0.05 were deemed statistically significant. A threshold of $|\log2FC| \geq 1$ (absolute log2 fold change) was imposed to ensure biological relevance and consistency across datasets, representing a two-fold or greater change in gene expression. For genes represented by multiple significant probes or probe sets, the probe with the lowest adjusted p-value was retained to represent that gene in downstream mito-DEG analysis. The resulting DEG lists for each dataset were subsequently used to identify mitochondrial gene signatures.

2.3. Identification of Mitochondria-Related Differentially Expressed Genes

To pinpoint mitochondrial genes involved in RA, we compared the significant DEG lists from each dataset against the MitoCarta 3.0 database (accessed April/ 2026). The Broad Institute compiled MitoCarta 3.0, a curated resource comprising 1,136 genes that code for mitochondrial proteins. This database also includes details regarding the sub-mitochondrial locations of these proteins and the biological pathways with which they are associated (Rath et al., 2021).

The intersection of the DEG lists with the MitoCarta 3.0 database was determined using standard set operations. Genes overlapping between the two sets were defined as mitochondria-related differentially expressed genes (mito-DEGs). To evaluate shared and tissue-specific mitochondrial alterations in RA, mito-DEGs identified from PBMC (GSE17755), synovial biopsy (GSE89408), and whole blood (GSE93272) datasets were compared using Venn diagram analysis. Venn diagrams were produced using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/; accessed April/2026), an online resource that supports the interactive comparison of up to four gene lists.

2.4. Construction of PPI Networks and Extraction of Hub Genes

To examine functional interactions between the mito-DEG we had identified, protein-protein interaction (PPI) networks were generated using the STRING database (version 12.0; accessed April/2026) (Szklarczyk et al., 2023). A minimum confidence score of 0.700 was required for interactions to be considered high-confidence, and only those with a combined score of ≥ 0.700 were retained for network construction. Following export, the interaction networks were transferred into Cytoscape software (version 3.10.1; accessed April/2026) for visualization and further topological analysis (Shannon et al., 2003). The identification of key hub genes within each network was accomplished using the Maximal Clique Centrality (MCC) algorithm as implemented in the cytoHubba plugin (v0.1; accessed April/2026) of Cytoscape (Chin et al., 2014). This algorithm evaluates genes according to their topological significance, giving preference to nodes that are part of many maximal cliques. Genes with the highest MCC scores were selected as hub genes for subsequent functional enrichment and regulatory network analyses.

2.5. GO and KEGG Pathways

The top 10 hub genes identified separately in PBMCs, synovial biopsies, and whole-blood tissues were subjected to functional annotation and pathway enrichment analyses using version 6.8 of the DAVID Bioinformatics Resources (accessed April/2026). DAVID is a well-established web-based platform that offers a broad range of functional annotation tools designed for analyzing large-scale gene lists (Sherman et al., 2022). We performed Gene Ontology (GO) enrichment analysis, prioritizing the Biological Process (BP) category, to explore the biological functions potentially associated with alterations in mitochondrial genes. In addition, we examined the Cellular Component (CC) and Molecular Function (MF) categories to generate a comprehensive functional profile of the hub genes (Ashburner et al., 2000).

To identify signaling and metabolic pathways linked to the mitochondrial hub genes, we conducted KEGG pathway enrichment analysis (Kanehisa et al., 2023). The Benjamini–Hochberg FDR method was applied for multiple testing correction, with an adjusted p-value < 0.05 considered significant. Bubble plots were generated to visualize the data; bubble size corresponds to the number of genes in each pathway, and color intensity reflects the significance level (log10 p-value).

2.6. Construction of Regulatory Networks

TF–gene and miRNA–gene interaction networks were constructed to explore the regulation of candidate hub genes. JASPAR (11th release, 2026), a curated open-access database of transcription factor binding profiles

across multiple species, served as the source for TF–gene interactions (Ovek Baydar et al., 2026; accessed May/2026). miRNA–gene interactions were retrieved from miRTarBase (version 9.0), a manually curated database that collects experimentally validated miRNA-target interactions (Huang et al., 2020; accessed May/2026).

NetworkAnalyst (a web-based tool for gene expression profiling and network visualization, version 3.2 as accessed in May/2026) was used to construct and visualize our networks (Xia et al., 2015; Zhou et al., 2019). We first created separate miRNA–mRNA and TF–mRNA networks, then merged them into a combined miRNA–TF–mRNA network to reveal the multilayered regulatory architecture controlling mitochondrial gene expression.

Key regulatory components within these networks were identified through topological analysis using metrics such as node degree (number of connections a node has) and betweenness centrality (a measure of a node's importance in connecting different parts of the network). These metrics were used to evaluate the relative importance of individual nodes (miRNAs, TFs, and genes) within the network. The top-ranked regulatory nodes were considered potential master regulators of mitochondrial dysfunction in RA.

3. Results

3.1. Identification of Differentially Expressed Genes in RA across Multiple Tissues

Differential gene expression analyses were performed on the PBMC (GSE17755), synovial biopsy (GSE89408), and whole blood (GSE93272) datasets using the GEO2R platform to investigate transcriptional alterations associated with RA.

PBMC analysis revealed 593 DEGs (268 up, 325 down) in RA samples compared to healthy controls. In the synovial biopsy dataset, a markedly larger number of transcriptional alterations were observed, with 9,161 DEGs, including 4,216 upregulated and 4,945 downregulated genes. Similarly, analysis of the whole blood dataset identified 1,878 DEGs, consisting of 1,112 upregulated and 766 downregulated genes in RA patients relative to controls.

3.2. Discovery of Mitochondria-Connected Differentially Expressed Genes

To specifically investigate mitochondrial involvement in RA, the identified DEGs from each dataset were intersected with the MitoCarta 3.0 database, which catalogues genes encoding mitochondrial proteins. This filtering approach identified 37 mitochondria-related DEGs (mito-DEGs) in the PBMC dataset, 220 mito-DEGs in the synovial biopsy dataset, and 119 mito-DEGs in the whole blood dataset.

Comparative analysis across tissues using Venn diagrams (Venny 2.1) was performed to identify shared and tissue-specific mitochondrial gene expression patterns. As shown in Figure 1, unique mito-DEGs were observed in PBMCs (22 genes), synovial biopsies (181 genes), and whole blood samples (82 genes). Shared genes were identified between PBMC and synovial biopsy (8 genes), PBMC and whole blood (6 genes), and synovial biopsy and whole blood (30 genes). Remarkably, only one gene (*AKAP1*) was found to be common across all three tissues in this dataset collection. The specific shared genes between PBMC and whole blood included *NDUFS4, PHB2, ABCB7, IVD, MRPS10, and SLC25A6;* between synovial biopsy and PBMC included *SOD2, PDK2, PRODH, GLRX5, AMT, ALAS2, MAOA, and BCL2A1;* and between synovial biopsy and whole blood included 30 genes *(SDHB, VDAC1, MRPL13, NDUFA5, MRPL3, COX7A2, TMEM126A, MRPL47, COQ2, PRELID3B, NDUFB3, MTHFD2, MPV17L, RH0T2, LYPLA1, MACR0D1, KMO, NDUFB4, TOMM5, ACSS1, TMEM126B, DBI, CMPK2, IFI27, FASTK, PMAIP1, CASP9, MCL1, BAD,* and *CASP3).*

3.3. Protein–Protein Interaction Network and Hub Gene Analysis

Hub genes were identified in mito-DEG PPI networks using the MCC algorithm in Cytoscape. In the PBMC dataset, the top 10 hub genes identified in the PPI network included *NDUFA10, ALAS2, TUFM, ABCB7, MRPS10, NDUFS4, SLC25A24, PHB2, HSPA9* and *NDUFV1*. In the synovial biopsy dataset, the top 10 hub gene analysis identified *UQCRQ, NDUFA5, NDUFA4, NDUFB4, NDUFB3, NDUFA12, UQCR10, NDUFV2, COX5A* and *NDUFS7* as highly connected nodes within the network. In the whole blood dataset, the PPI network analysis revealed the top 10 hub genes, including *NDUFA11*, *UQCRFS1*, *NDUFB3*, *NDUFS6*, *NDUFS4*, *UQCRC1*, *NDUFB4*, *COX7A2*, *NDUFS2* and *NDUFA5*. The PPI networks of the identified hub genes for PBMC, synovial biopsy, and whole blood datasets are presented in Figure 2.

3.4. GO and KEGG Pathways

GO and KEGG pathway enrichment analyses for the mitochondrial hub genes identified in each dataset were carried out using DAVID Bioinformatics Resources (version 6.8). The enrichment results are presented as bubble plots in Figure 3.

PBMC dataset: Enriched BP terms included "mitochondrial electron transport from NADH to ubiquinone", "proton transmembrane transport", "proton motive force-driven mitochondrial ATP synthesis", "aerobic respiration", and "iron-sulfur cluster assembly". CC terms included "mitochondrial inner membrane", "mitochondrial matrix", and "respiratory chain complex I". MF categories included "NADH dehydrogenase(ubiquinone)activity and oxidoreductase activity". KEGG pathway enrichment included "oxidative phosphorylation", "thermogenesis", "chemical carcinogenesis-reactive oxygen species pathways", and "pathways of neurodegeneration-multiple diseases" (Figure 3A).

Synovial biopsy dataset: Enriched BP terms included "proton transmembrane transport", "mitochondrial electron transport from NADH to ubiquinone", "aerobic respiration", "mitochondrial electron transport from

ubiquinol to cytochrome c", and "mitochondrial respiratory chain complex I assembly". CC terms included "mitochondrial inner membrane", "respiratory chain complex I", and "mitochondrion". MF terms included "NADH dehydrogenase (ubiquinone) activity", "oxidoreductase activity", and "iron-sulfur cluster binding". KEGG pathways included "oxidative phosphorylation", "non-alcoholic fatty liver disease", "thermogenesis" and "chemical carcinogenesis-reactive oxygen species" (Figure 3B).

Whole blood dataset: Enriched BP terms included "aerobic respiration", "proton transmembrane transport", "mitochondrial electron transport from NADH to ubiquinone", "mitochondrial respiratory chain complex I assembly", and "oxidative phosphorylation". CC terms included "mitochondrial inner membrane", "respiratory chain complex I", and "mitochondrion". MF terms included "NADH dehydrogenase(ubiquinone)activity", "quinol-cytochrome-c reductase activity", "oxidoreductase activity", and "iron-sulfur cluster binding". KEGG pathway enrichment included "oxidative phosphorylation", "non-alcoholic fatty liver disease", "chemical carcinogenesis-reactive oxygen species", and "thermogenesis" (Figure 3C).

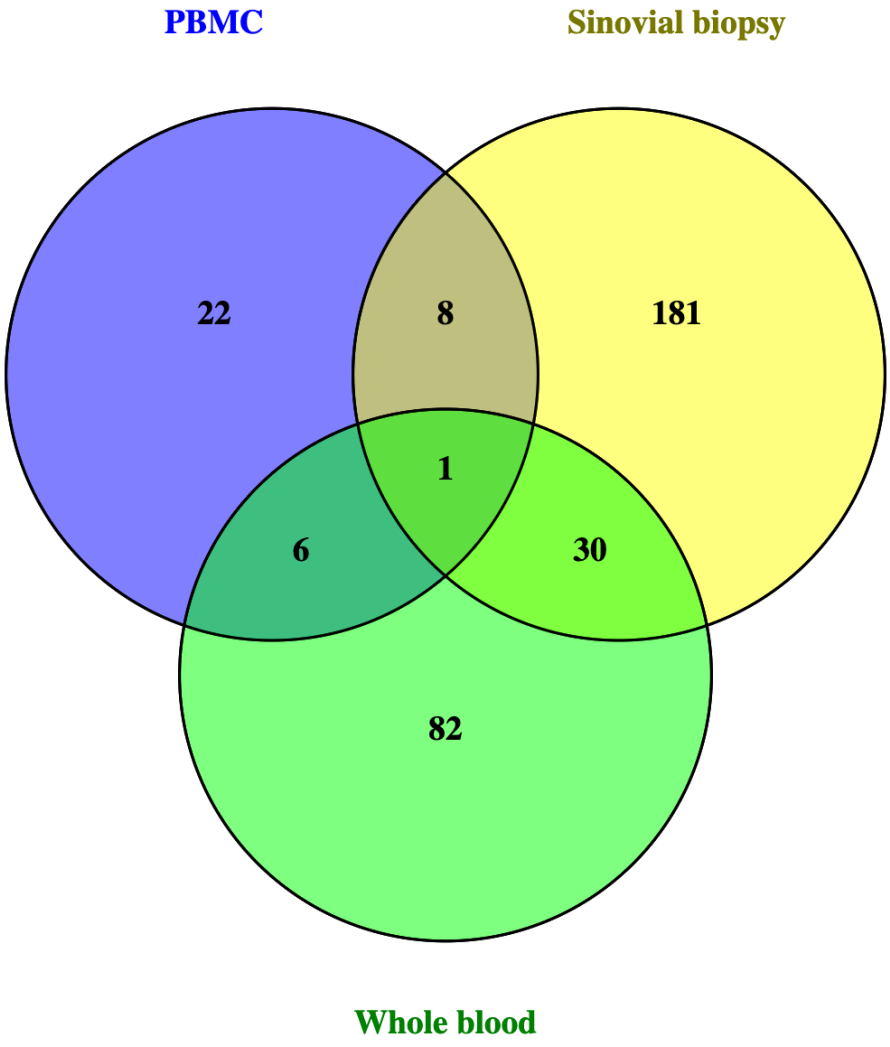

Figure 1. Venn diagram showing the distribution of mitochondria-related differentially expressed genes (mito-DEGs) across PBMC, synovial biopsy, and whole blood datasets in rheumatoid arthritis. The diagram illustrates the overlap of mito-DEGs identified from the PBMC (GSE17755), synovial biopsy (GSE89408), and whole blood (GSE93272) datasets after intersecting the differentially expressed genes with the MitoCarta 3.0 database. Unique mito-DEGs were observed in PBMC (22 genes), synovial biopsy (181 genes), and whole blood (82 genes). Shared genes were identified between PBMC and synovial biopsy (8 genes), PBMC and whole blood (6 genes), and synovial biopsy and whole blood (30 genes). One mito-DEG was found to be common across all three datasets.

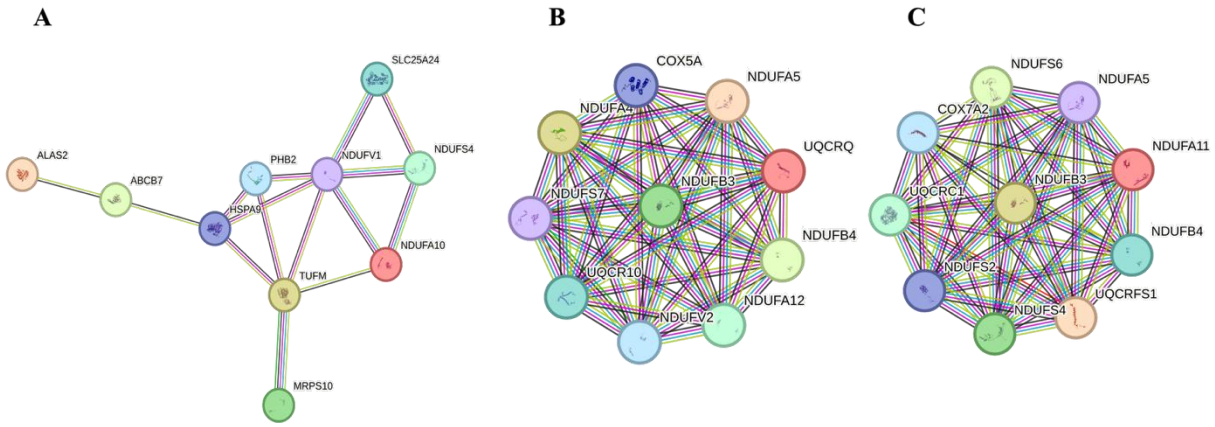

Figure 2. Protein–protein interaction (PPI) networks of mitochondrial hub genes identified in different tissues. The networks represent the top 10 hub genes identified using the MCC algorithm in cytoHubba for (A) PBMC, (B) synovial biopsy, and (C) whole blood datasets. Nodes represent genes and edges indicate predicted functional interactions derived from the STRING database.

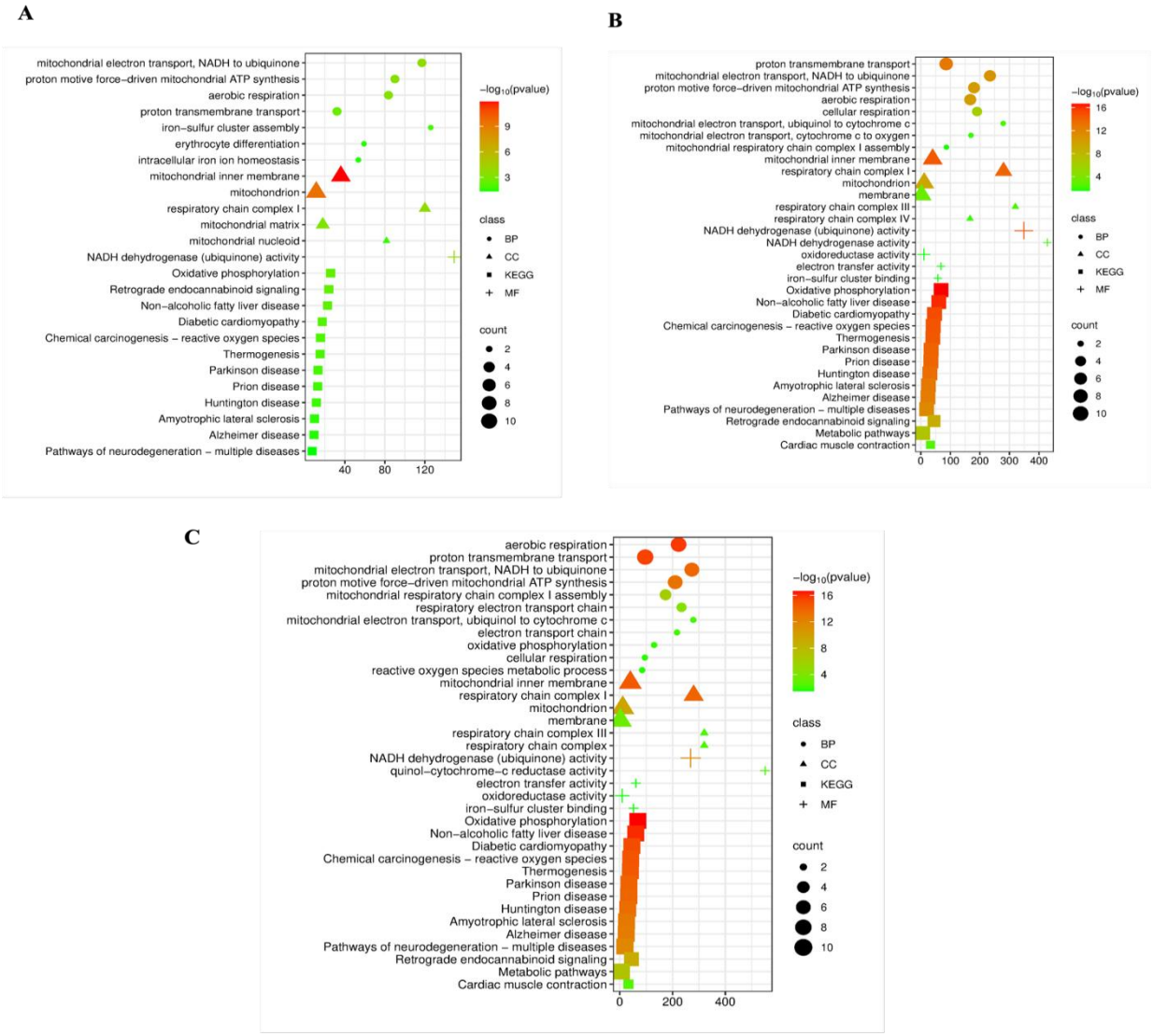

Figure 3. Functional enrichment analysis of mitochondrial hub genes across different tissues in rheumatoid arthritis. Bubble plots showing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment results for the top hub genes identified in (A) PBMC, (B) synovial biopsy, and (C) whole blood datasets. The y-axis represents enriched GO terms and KEGG pathways, while the x-axis indicates gene counts associated with each term. Bubble size corresponds to the number of genes involved in each pathway, and color intensity represents the significance level expressed as –log10(P-value).

3.5. Regulatory Network Topology of Candidate Hub Gene Biomarkers

To characterize the regulatory interactions associated with candidate hub genes, miRNA–mRNA, TF–mRNA, and integrated miRNA–TF–mRNA regulatory networks were constructed for PBMC, synovial biopsy, and whole blood datasets (Figures 4–6). A complete list of miRNAs and TFs targeting the identified mitochondrial hub genes, along with their interaction frequencies, is provided in Supplementary Table 1.

In the PBMC dataset, the miRNA–mRNA interaction network revealed that several miRNAs targeted multiple hub genes. Two miRNAs, "hsa-miR-16-5p" and "hsa-miR-615-3p", each targeted three hub genes, while forty miRNAs targeted two hub genes. In the TF–mRNA network, FOXC1 was identified as the transcription factor interacting with nine hub genes. GATA2 was associated with five hub genes, whereas POU2F2 and NFIC were each linked to four hub genes (Figure 4).

In the synovial biopsy dataset, the regulatory network analysis identified "hsa-miR-26-5p" as the miRNA targeting the highest number of hub genes (four). Six miRNAs ("hsa-miR-149-3p", "hsa-miR-4728-5p", "hsa-miR-6785-5p", "hsa-miR-6883-5p", "hsa-miR-1226-3p", and "hsa-miR-484") were each associated with two hub genes. In the TF–mRNA network, FOXC1 targeted five hub genes, and PPARG targeted four. Additionally, seven transcription factors (TP53, USF2, MEF2A, CREB1, STAT3, TP63, and E2F1) were each connected to three hub genes (Figure 5).

In the whole blood dataset, the miRNA–mRNA network identified five miRNAs ("hsa-miR-92a-3p", "hsa-miR-8485", "hsa-miR-3173-3p", "hsa-miR-6891-5p", and "hsa-miR-26b-5p"), each targeting two hub genes. In the TF–mRNA network, three TFs (YY1, FOXC1, and GATA2) were each associated with five hub genes. POU2F2 and FOXL1 were linked to four hub genes (Figure 6).

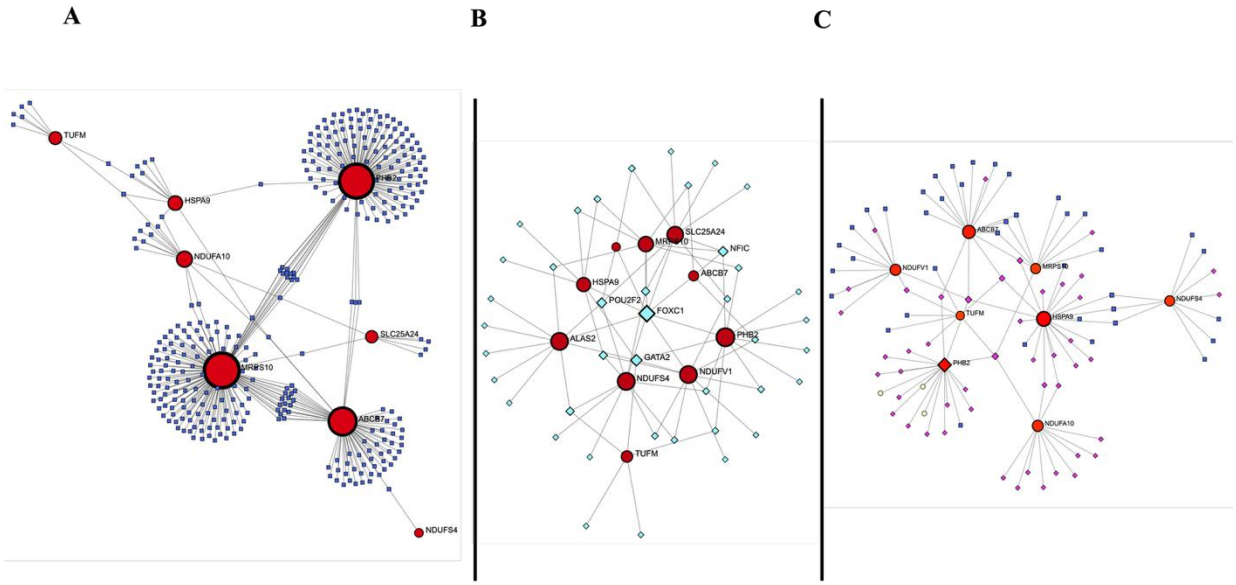

Figure 4. Regulatory interaction networks of mitochondrial hub genes in PBMC samples from rheumatoid arthritis. (A)miRNA–hub gene regulatory network showing interactions between microRNAs and mitochondrial hub genes identified in the PBMC dataset. (B) Transcription factor (TF)–hub gene regulatory network illustrating predicted TF–gene interactions associated with the identified mitochondrial hub genes. (C) Integrated TF–miRNA–hub gene regulatory network combining transcription factors, microRNAs, and hub genes to reveal the potential multilayered regulatory architecture controlling mitochondrial gene expression in PBMC samples.

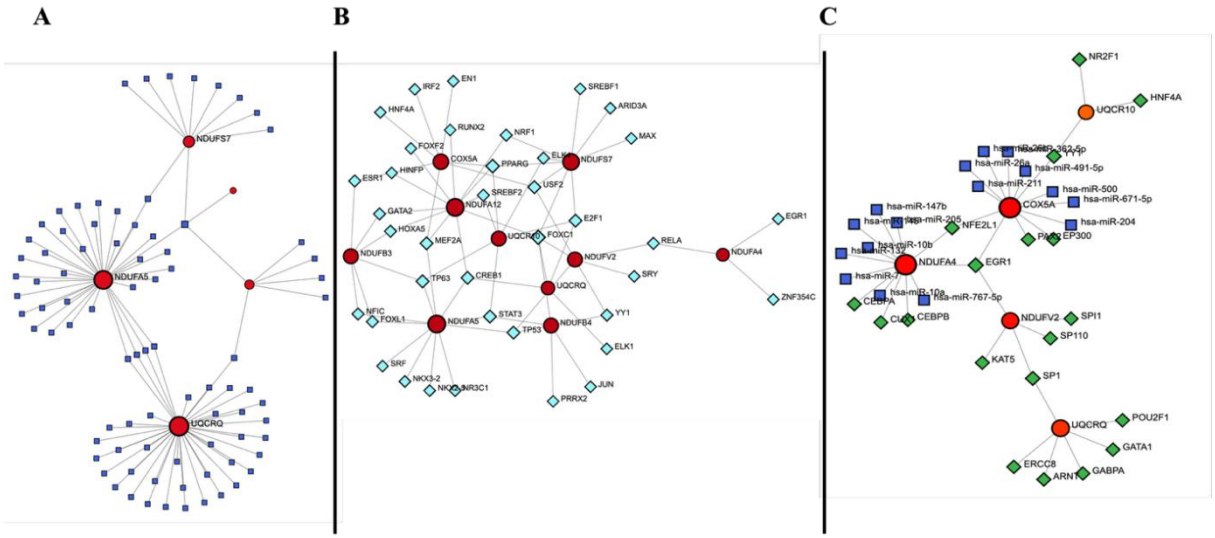

Figure 5. Regulatory interaction networks of mitochondrial hub genes in synovial biopsy samples from rheumatoid arthritis. (A)miRNA–hub gene regulatory network showing interactions between microRNAs and mitochondrial hub genes identified in the synovial biopsy dataset. (B) Transcription factor (TF)–hub gene regulatory network illustrating predicted TF–gene interactions associated with the identified mitochondrial hub genes. (C) Integrated TF–miRNA–hub gene regulatory network combining transcription factors, microRNAs, and hub genes to reveal the potential multilayered regulatory architecture controlling mitochondrial gene expression in synovial biopsy samples.

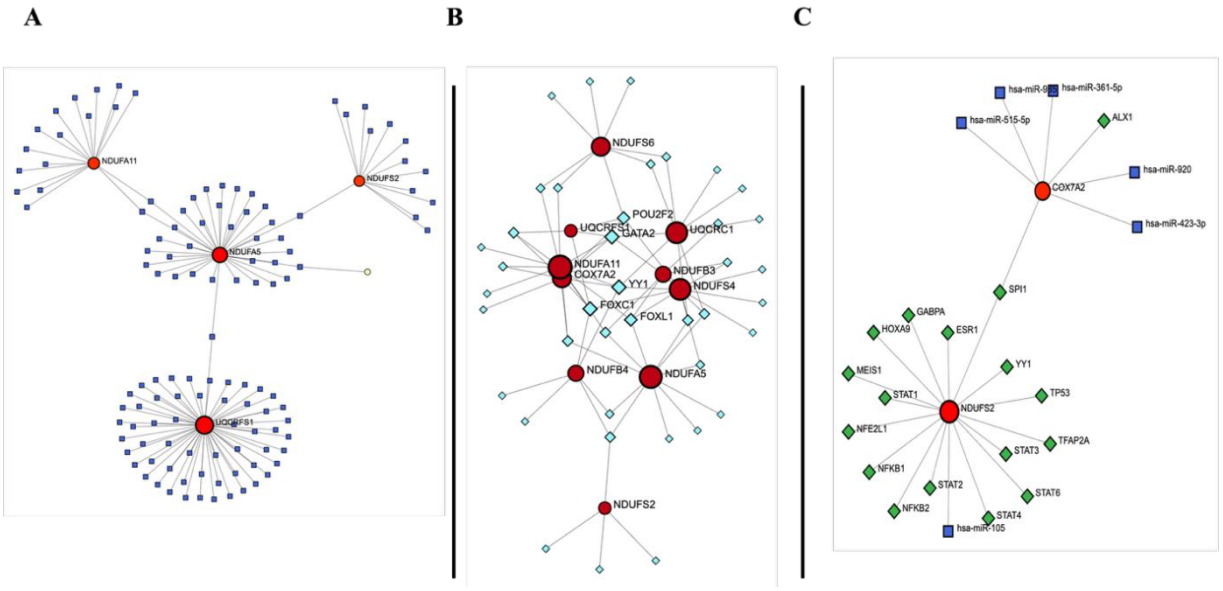

Figure 6. Regulatory interaction networks of mitochondrial hub genes in whole blood samples from rheumatoid arthritis. (A)miRNA–hub gene regulatory network showing interactions between microRNAs and mitochondrial hub genes identified in the whole blood dataset. (B) Transcription factor (TF)–hub gene regulatory network illustrating predicted TF–gene interactions associated with the identified mitochondrial hub genes. (C) Integrated TF–miRNA–hub gene regulatory network combining transcription factors, microRNAs, and hub genes to reveal the potential multilayered regulatory architecture controlling mitochondrial gene expression in whole blood samples.

4. Discussion

RA is a complicated autoimmune disease driven by long-term synovial inflammation and systemic immune abnormalities (Smolen et al., 2016). Despite the growing recognition of mitochondrial dysfunction as a key player in RA pathogenesis (Fearon et al., 2016), most previous transcriptomic studies have focused on single tissue types (Lee et al., 2020), limiting our understanding of shared versus tissue-specific mitochondrial alterations. In this study, we performed an integrative transcriptomic analysis of three distinct biological compartments—PBMCs, synovial biopsies, and whole blood samples—to systematically characterize mitochondrial gene expression signatures in RA. Our multi-tissue approach revealed both common and tissue-specific mitochondrial dysregulation, with oxidative phosphorylation (OXPHOS)

components emerging as central hub genes across all compartments, and *AKAP1* identified as the only mitochondrial gene common to all three tissues in this dataset collection.

The number of mito-DEGs varied considerably across tissues, with synovial biopsy showing the highest number (220 mito-DEGs), followed by whole blood (119 mito-DEGs) and PBMC (37 mito-DEGs). This gradient likely reflects the severity of local inflammation in the joint microenvironment. The primary inflammatory site in RA, synovial tissue faces hypoxia, pro-inflammatory cytokines (TNF-α, IL-6, IL-17), and oxidative stress, all of which directly impair mitochondrial function (Mihaylova et al., 2024). In contrast, PBMC and whole blood represent systemic compartments where mitochondrial alterations may be more moderate but still detectable, consistent with the concept of RA as a systemic disease (Abbasifard et al., 2024; Mitrović et al., 2023). It should also be briefly noted that this gradient may partly reflect platform differences, since GSE17755 was profiled on the Hitachisoft GPL1291 platform, GSE93272 on the Affymetrix GPL570 platform, and GSE89408 on the Illumina GPL11154 platform, and these technical differences may contribute to some of the variation in DEG/mito-DEG numbers observed between synovial biopsy and the other two tissues, independent of true biological differences.

Remarkably, *AKAP1* was the only mitochondrial gene commonly dysregulated across all three tissues in this dataset collection. *AKAP1* serves as a mitochondrial scaffold protein that tethers protein kinase A (PKA) to the outer mitochondrial membrane, where it regulates multiple mitochondrial functions, including oxidative phosphorylation, mitochondrial dynamics (fission/fusion balance), and apoptosis (Liu et al., 2020). *AKAP1* has been shown to prevent oxidative mitochondrial damage by promoting mitophagy and maintaining membrane potential (Soundararajan et al., 2022). To our knowledge, this is the first study to identify AKAP1 as a common mitochondrial signature across multiple tissues in RA, raising the possibility that it may serve as a systemic biomarker of mitochondrial dysfunction; however, this remains speculative pending independent validation. Future studies should evaluate the diagnostic performance of *AKAP1* using ROC curve analysis in independent validation cohorts.

Across tissues, hub genes were predominantly Complex I subunits (NADH:ubiquinone oxidoreductase), including multiple NDUF genes. Complex I is the largest and most complex ETC enzyme, initiating NADH-to-ubiquinone electron transfer and proton pumping across the inner mitochondrial membrane (Bozdemir et al., 2025).

Dysregulation of Complex I subunits has previously been implicated in RA. Fearon et al. showed that reduced complex I activity and increased ROS production in RA synovial fibroblasts promote their invasiveness (Fearon et al., 2016). Similarly, a recent study by Huang et al. showed that peripheral blood mitochondrial DNA gene variation is associated with RA susceptibility (Huang et al., 2026). Our study extends these findings by demonstrating that complex I subunit dysregulation is not limited to synovial tissue but is also detectable in peripheral compartments (PBMC and whole blood), suggesting that OXPHOS impairment is a systemic feature of RA. Notably, *NDUFB4* and *NDUFB3* were identified as hub genes in both synovial biopsy and whole blood datasets, making them potential blood-based biomarkers of synovial mitochondrial dysfunction.

KEGG enrichment analysis showed that mitochondrial hub genes were significantly enriched in oxidative phosphorylation, thermogenesis, and several neurodegenerative disease pathways (Parkinson's, Alzheimer's, Huntington's, ALS, and prion disease). This intriguing finding suggests that shared mitochondrial stress signatures may underlie both RA and neurodegenerative disorders.

Accumulating evidence supports a link between chronic inflammation and neurodegeneration. Systemic inflammation has been shown to accelerate cognitive decline and increase the risk of neurodegenerative diseases (Jaramillo Ramos et al., 2025). Mitochondrial dysfunction, defined by impaired OXPHOS, elevated ROS production, and defective mitophagy, is a common pathogenic mechanism in both RA and neurodegenerative diseases (Ma et al., 2022). Our results raise the possibility that mitochondrial hub genes identified in RA patients may represent shared therapeutic targets for inflammation-associated neurodegeneration. The enrichment of thermogenesis pathways is also notable, as uncoupling proteins (UCPs) in mitochondria regulate ROS production and inflammatory responses in immune cells (Nesci and Rubattu, 2024).

Our regulatory network analysis identified FOXC1 and GATA2 as transcription factors interacting with multiple hub genes across all three tissues, suggesting that they may act as master regulators of mitochondrial gene expression in RA.

FOXC1 (Forkhead Box C1) is a transcription factor involved in cellular stress responses and has been shown to promote the expression of antioxidant genes, protecting cells from oxidative damage (Berry et al., 2008). In the context of RA, FOXC1 may represent a compensatory response to increased oxidative stress in inflamed tissues. GATA2 is a hematopoietic transcription factor that regulates the expression of mitochondrial genes in macrophages and dendritic cells, influencing their metabolic reprogramming during inflammation (Jackson-Strong et al., 2026). Our finding that GATA2 targets multiple mitochondrial hub genes in PBMC and whole blood suggests that GATA2-mediated transcriptional regulation may contribute to the metabolic adaptation of immune cells in RA.

The miRNA “hsa-miR-16-5p” emerged as a key post-transcriptional regulator, targeting three hub genes in PBMC. Huang et al. recently demonstrated that

peripheral blood miR-16-5p levels are altered in RA patients and correspond with disease activity (Huang et al., 2026). Our results suggest that miR-16-5p may exert its effects in part by regulating mitochondrial gene expression. These regulatory nodes may represent novel therapeutic targets for restoring mitochondrial function in RA.

Our findings are consistent with and extend previous transcriptomic studies in RA. In a recent study, Tariq et al. applied an integrated multi-omics method to uncover potential therapeutic biomarkers in RA and also identified mitochondrial genes among their candidates (Tariq et al., 2025). A 2025 study investigating mitochondrial apoptosis-related genes (MARGs) in RA identified hub genes including MRPS10 and TUFM (which we also identified in PBMC) and demonstrated their diagnostic potential through ROC analysis, with transcription factors such as YY1 and FOXO3 emerging as critical regulators (Gao et al., 2025). Notably, YY1 was also identified as a key transcription factor in our whole blood dataset, consistent with this previous report.

Lee et al. revealed distinct inflammatory pathways in blood versus arthritic joints, emphasizing the need for multi-tissue approaches in biomarker discovery (Lee et al., 2020). Our study directly addresses this limitation by integrating data from three distinct compartments and identifying both shared and tissue-specific mitochondrial signatures. Additionally, a recent study demonstrating that the MTHFD2-CKMT1 axis promotes osteoclastogenesis and bone loss through enhanced OXPHOS activity further supports the central role of oxidative phosphorylation in RA pathogenesis (Li et al., 2025). Mitochondrial dysfunction not only impairs oxidative phosphorylation but also sensitizes cells to intrinsic apoptotic signals, and the modulation of these pathways has been extensively investigated in the context of targeted cancer and autoimmune therapies (Gökhan et al., 2020).

Our findings have several potential clinical implications. First, the candidate genes identified in this study—particularly *AKAP1* and the OXPHOS subunit genes *NDUFB4* and *NDUFB3*—merit investigation as potential blood-based biomarkers of mitochondrial dysfunction in RA in future prospective studies. If validated in independent cohorts, they could offer a minimally invasive approach for patient stratification and monitoring. Second, the identification of FOXC1, GATA2, and miR-16-5p as master regulators suggests that modulating these regulatory nodes could restore mitochondrial function in RA. Third, our results support the rationale for developing mitochondria-targeted therapies (such as MitoQ or MitoTEMPO) or repurposing existing drugs like metformin for RA treatment (Hou et al., 2025).

Some limitations should be noted. Two of the three datasets analyzed (GSE17755, GSE93272) were microarray-based, which have lower sensitivity and dynamic range than RNA-seq; validation using RNA-seq data from additional independent cohorts is still needed to confirm the microarray-derived findings. A related limitation concerns the mixed-platform nature of our dataset collection: GSE17755 was profiled on the Hitachisoft GPL1291 two-color oligo array, GSE93272 on the single-color Affymetrix GPL570 platform, and GSE89408 was generated by Illumina GPL11154 RNA sequencing rather than by microarray. Because each dataset was analyzed independently, with DEG lists compared only at the level of shared gene identity (Venn diagram) rather than by merging normalized expression values, we did not apply cross-platform batch correction. Platform-specific differences in probe design, sensitivity, and dynamic range across the two microarray platforms, together with more fundamental technology-level differences between microarray and RNA-sequencing for GSE89408 — including sensitivity to low-abundance transcripts and the underlying statistical testing framework (voom-transformed counts vs. array-based linear models) — may contribute to the substantial disparity in DEG and mito-DEG counts observed between the synovial biopsy dataset and the other two datasets, independent of true biological differences between tissues. This limits direct quantitative comparison of DEG magnitude across compartments, although qualitative overlap in specific genes remains informative. Relatedly, for the two microarray datasets, differential expression was tested at the probe level, and genes with multiple significant probes were collapsed to a single entry using the lowest adjusted *p*-value rule described in Section 2.2. A different probe-collapsing convention (e.g., selecting the probe with the highest mean expression) could, in principle yield marginally different DEG counts. Additionally, the uniform $|\log2FC| \geq 1$ threshold applied across all three datasets may be comparatively stringent for circulating immune cells, where transcriptional changes are often more modest than in locally inflamed tissue, potentially contributing to the lower mito-DEG count observed in the PBMC dataset relative to synovial biopsy. A lower-threshold or rank-based sensitivity analysis in PBMC-specific data represents a valuable direction for follow-up work. Finally, gene expression changes do not always correlate with protein levels or enzymatic activity. This is a particularly relevant caveat for our findings, since none of the candidate genes highlighted in this study (including AKAP1 and the NDUF family hub genes) have been validated at the protein or enzymatic activity level in samples from RA patients; targeted validation using methods such as Western blotting, immunohistochemistry, or activity assays of OXPHOS complex I is needed before these observed transcriptional changes can be interpreted as reflecting altered mitochondrial protein abundance or function. Our study design is also descriptive and correlative rather than mechanistic and cannot establish whether the observed mitochondrial transcriptional changes are drivers of RA pathogenesis, consequences of the inflammatory environment, or both. Functional

experiments — for example, CRISPR-Cas9-mediated knockout or knockdown of AKAP1 or NDUF-family genes in immune or synoviocyte cell models, or pharmacological modulation of oxidative phosphorylation — would be needed to establish causal relationships and would represent an important direction for future work. A further limitation is the potential clinical heterogeneity among the three GEO cohorts used in this study. The original dataset does not provide harmonized information on patient age, sex distribution, disease duration, disease activity, or treatment status (e.g., DMARD-naïve versus treated), and these variables were therefore neither available nor controlled for in our analysis. Differences in these clinical characteristics across the PBMC, synovial biopsy, and whole blood cohorts could contribute to some of the tissue-level differences we observed, independent of the biological compartment itself, and this should be considered when interpreting cross-tissue comparisons. Finally, bulk tissue analyses mask cellular heterogeneity: PBMC, whole blood, and synovial biopsy each consist of multiple cell types (e.g., synovial fibroblasts, macrophages, T and B lymphocytes) with distinct mitochondrial profiles, and bulk expression values represent averages across this mixture. Single-cell or spatial transcriptomic studies are needed to resolve which specific cell populations drive the mitochondrial signatures identified here, particularly within the synovial compartment where cellular heterogeneity is likely greatest.

5. Conclusion

This multi-tissue integrative analysis reveals that mitochondrial dysfunction in rheumatoid arthritis is tissue-specific yet converges on oxidative phosphorylation dysregulation, with synovial tissue showing the most extensive transcriptional alterations and Complex I (NDUF family) subunits emerging as central hub genes across all three compartments. AKAP1 emerges as a candidate systemic signature requiring independent validation, while FOXC1, GATA2, and hsa-miR-16-5p are identified as key transcriptional and post-transcriptional regulatory nodes. By jointly profiling PBMC, synovial biopsy, and whole blood within a single analytical framework, this study helps distinguish systemic from tissue-restricted mitochondrial signatures in RA. These findings provide a resource for future studies investigating mitochondrial contributions to RA pathogenesis and for developing diagnostic or therapeutic strategies targeting mitochondrial pathway.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	S.A.
C	100
D	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100

C= concept, D= design, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required because this study used only publicly available, de-identified transcriptomic datasets.

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