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Exploring the transcriptomic landscape of rheumatoid arthritis using next-generation RNA sequencing

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation and joint damage. Transcriptomics has been utilized in RA profiling to identify locations that were previously unclear. A robust technique for examining the intricate transcriptomic landscape of RA is next-generation RNA sequencing (RNA-seq). This review has explored the use of RNA-seq to understand the pathophysiology of RA. Detailed discussions are presented on the fundamentals of RNA-seq technology, how RNA extracted from the synovium is incorporated into gene expression patterns analysis, and how RNA-seq is superior to other methods. We have explored several applications of RNA-seq in RA research, emphasizing its potential for identifying dysregulated pathways, detecting novel biomarkers, and characterizing gene expression levels. Furthermore, this review has clarified the regulatory networks and signaling pathways identified in RNA-seq research and investigated the possibility of using transcriptomic RNA-seq as a diagnostic tool for RA. In conclusion, we have highlighted the significance of using transcriptome data to achieve a more comprehensive understanding of the molecular processes underlying RA. Hence, this review underscores the revolutionary influence of RNA-seq on RA research, paving the way for improved diagnostics, tailored therapy, and the discovery of novel treatment targets for individuals with RA.

Keywords: Rheumatoid arthritis; Next-generation RNA sequencing; Long non-coding RNA; Circular RNA; Transcriptomic study

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1. Introduction

Approximately 0.5 – 1.0% of people worldwide suffer from rheumatoid arthritis (RA), a prototypical chronic inflammatory rheumatic disease that is affected by various hereditary, epigenetic, and environmental factors. RA is a prevalent autoimmune disease that affects the entire body and is characterized by significant joint dysfunction and persistent inflammation. The source of the original term "rheumatoid arthritis" indicates inflamed and fluid joints. Although RA primarily affects the joints, it may impact other organ systems such as the eyes, skin, lungs, liver, heart, and bones. Symptoms of RA often include pain, stiffness, fever, edema, and inflammation in the afflicted joints. These symptoms generally affect the small joints in the hands and feet; however, they can also

affect larger joints such as the knees and shoulders. Increased stiffness in the morning is a prominent symptom of this condition, and these symptoms become more noticeable after extended periods of inactivity.[1-3] RA-related pain is categorized as nociceptive rather than neuropathic because it originates at the site of inflammation. As the pathological state worsens, continued inflammation causes tendon binding, erosion, and degradation of the articular surface; this can limit the range of motion and cause deformity and local osteoporosis, which frequently develops around the inflamed joints of patients with RA.[4] Rheumatoid and musculoskeletal disorders represent a significant global economic burden, and their prevalence is increasing because of changes in behavior and population; among them, RA is the most prevalent inflammatory arthritis. As these disorders cause more functional impairments in adults than any other ailments, they are considered the primary cause of disability and early retirement among workers. Therefore, early identification and individualized, cell-specific therapies are essential for the management of RA.[5]

1.1. Pathophysiology of RA

In RA, the synovium is essentially inflamed, which causes erosion of the bone and cartilage, as depicted in Figure 1. Although the precise cause of the inflammation remains unknown, macrophages, which are often found in the synovium, are known to secrete cytokines such as tumor necrosis factor-α, interleukin-1, and interleukin-6, which cause inflammation. These cytokines stimulate fibroblast-like synoviocytes (synovial intimal cells are believed to be in charge of producing synovial fluid components), which start multiplying upon activation.[6] Simultaneously, synoviocytes support the expression of RANKL (osteoclast differentiation factor), which, in conjunction with cytokines, increases osteoclast activity (bone degradation), ultimately resulting in bone erosion.[7] Proteases are secreted by activated, proliferating synovial cells and tissues similar to fibroblasts. These proteases lead to cartilage deterioration or breakdown. Activation of fibroblast-like synoviocytes results in symmetrical arthritis. T cells can release interleukin-17, which activates fibroblast-like synoviocytes and increases macrophage activity; moreover, these cells induce inflammation.[8] Furthermore, T cells aid in the expression of RANKL, which activates osteoclasts to cause bone degradation. Moreover, plasma cells are present in this region; although plasma cells constitute only 5% of the overall immune cells, they support inflammation by producing cytokines and antibodies. Neutrophils are also present in the synovial fluid and secrete proteases and reactive oxygen species, which degrade cartilage and bone.[5,8]

An attribute of RA is the presence of immune complexes in the synovial fluid, which are composed of bonded antibodies that increase inflammation. All these cells release cytokines, which aid in increasing capillary permeability and adhesion molecule expression on the vascular vasculature, enabling immune cells to travel into the joint and induce RA, as shown in Figure 2.[9]

RA can be caused by factors such as smoking, heredity, and the bacterium *Porphyromonas gingival* that causes gingivitis. These factors can alter autoantigens, resulting in joint inflammation and cytokines. Antigen-presenting cells recognize this change and initiate an immune response. CD4 T-cell activation triggers B-cell activation in the germinal center, leading to the formation of plasma cells that generate autoantibodies against the body's own antigen.

An important component of RA is antibodies. Rheumatoid factor IgM antibody targets the Fc region of IgG and is present in 75% of patients with RA, as well as anticitrullinated protein antibodies, which target citrullinated proteins such as fibrin and filaggrin. These antibodies also cause inflammation and synovial fluid deposition. Proteins in which their arginine residues are citrullinated are known as citrullinated proteins. After the attack, the body targets this protein, recognizes it as a foreign particle, and begins to attack, causing swelling and inflammation.[5]

1.2. Clinical manifestation

RA is a symmetrical arthritic joint with pain, swelling, and nodules. Early symptoms affect the hand, particularly the proximal and metacarpal phalangeal joints. As the disease worsens, additional hand characteristics such as swan neck, Boutonniere, and thumb Z deformity appear. Angiogenesis, the expansion of blood vessels from pre-existing vasculature, is also observed in patients with RA.

2. Overview of transcriptomics: Understanding RNA sequencing (RNA-seq) technologies

Genomes in organisms are composed of DNA and genes, which remain almost identical in all cells. Nevertheless, when an organism experiences specific environmental changes, its cells must decode this genomic information differentially. This information is translated into RNA, which is used to produce proteins. The transcriptome is a complete set of messenger RNA (mRNA) transcribed in a cell at a specific time, expressing a part of the functional genome. Transcriptomic studies examine the transcriptome using various techniques.[10]

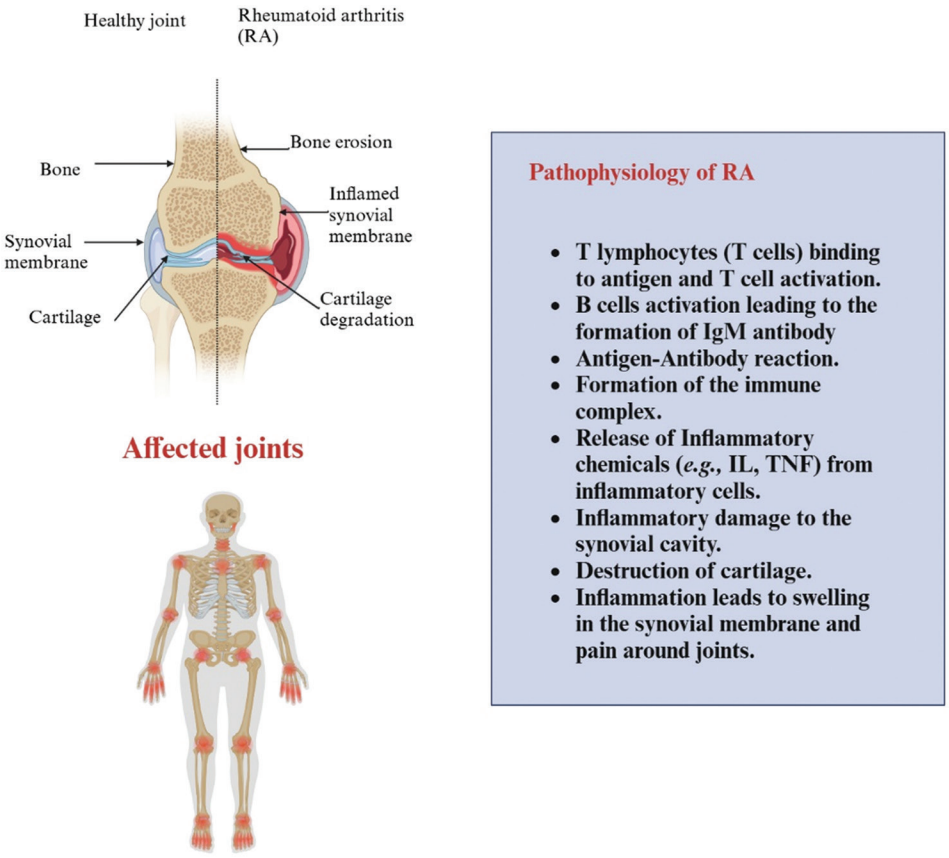


Figure 1. Pathophysiology of rheumatoid arthritis. Figure created by the author.
Abbreviations: RA: Rheumatoid arthritis; IL: Interleukin; TNF-α: Tumor necrosis factor-α.

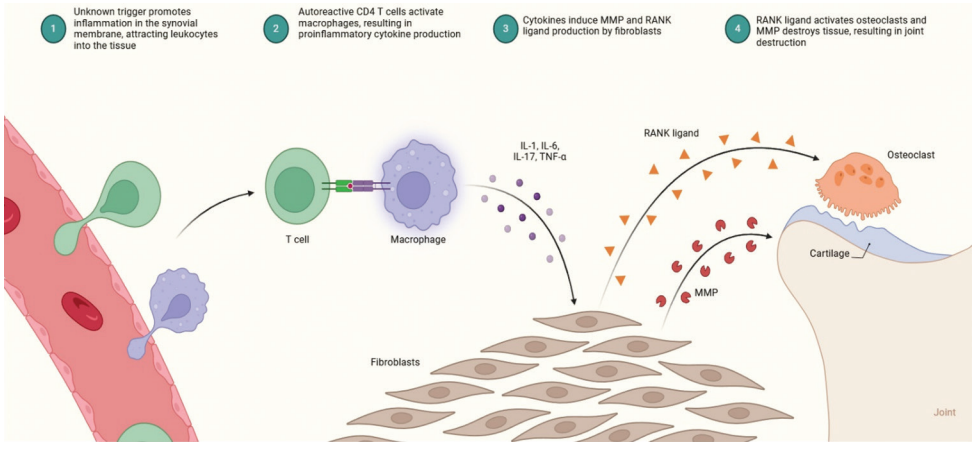


Figure 2. Pathogenesis of rheumatoid arthritis. Figure created by the author.
Abbreviations: IL: Interleukin; TNF-α: Tumor necrosis factor-α; MMP: Matrix metalloproteinase; RANK: Osteoclast differentiation factor.

The DNA microarray or chip method is based on nucleic acid hybridization. In microarrays, which are also known as cDNA arrays, a pre-determined set of probes representing the sequence of the gene fragments are fixed on a solid chip. A major limitation of this technique is that we must be aware of the gene sequence for designing the chip. Hence, for those species whose genomic sequence information is unknown, microarray is not a good option because chips are not readily available and the manufacturing of new chips is expensive. Another issue

with this array is cross-hybridization. Using full-length cDNAs, the mRNAs of paralogs can cross-hybridize to the wrong spots. A major challenge in analyzing microarray data is that it can be difficult to compare transcripts.[11]

Another method is a sequencing-based technique or RNA-seq. This technique largely replaces the microarrays, becoming popular for performing transcriptomics. The development of nanotechnology enables the sequencing of hundreds of thousands of DNA molecules simultaneously. RNA sequencing is a process for determining the nucleotide sequence of an RNA molecule in a specific sample. The primary principle is based on a high-output sequencing technique with a computational technique to capture and quantify transcripts present in an RNA extract. The nucleotide sequences generated are typically approximately 100 bp in length, but they can range from 30 bp to more than 10,000 bp, depending on the sequencing method used. In this method, RNA can first be isolated from biological samples and converted into double-stranded DNA molecules that serve as a template for DNA polymerases during next-generation sequencing (NGS) library preparation. These DNA copies are known as complementary DNA (cDNA), and the generation of cDNA is initiated using the reverse transcriptase enzyme and priming to form the first strand of cDNA.

The second strand is synthesized using DNA polymerase, leading to the formation of double-stranded cDNA. In the next step known as adapter ligation, the adapters ligated to the cDNA fragments and the resulting library molecules are amplified and sequenced.[12] In this method, short-read sequencing and map construction of the reads using a reference genome are performed using tools such as HISAT2 or STAR, and novel transcripts are assembled using tools such as StringTie. Other tools such as HTseq help in estimating the expression levels of the transcripts. Three factors, namely transcript level, transcript length, and sequencing depth, affect the number of read transcripts that can be detected in a sample. RNA-seq does not require prior genomic information, which makes it suitable for analyzing transcriptomes in non-model species.[13,14] Transcriptome analysis is generally performed for two major purposes; one is qualitative estimation, that is, identification of genes and their isoforms, and the other is quantitative analysis, that is, estimating the gene expression or relative expression in different biological samples.[15] RNA is less stable than DNA; however, the transcriptome is considerably more cell- and tissue-specific than the genome and is crucial for the generation of high-quality data.[16] Table 1 shows the transcriptomes used for RNA-seq to demonstrate the relevance of the approach across several conditions beyond RA.

Human leukocyte antigen (HLA) genes and non-HLA genes are the genes and genetic loci that have been linked to the risk of RA. RNA-seq sheds insights into the causes of RA and its therapy, making it easier to distinguish healthy individuals from those with RA. Studies[27,28] comparing population transcriptomes by analyzing the synovium, synovial cells, white blood cells, and peripheral blood mononuclear cells have been conducted. Studies have also revealed that patients with RA have dysregulated levels of secretory phosphoprotein 1 (SPP1). Inflammation, T-cell activation, and apoptosis are caused by SPP1. Proteoglycan 4 is detected in the severe stage of RA. According to some studies, a correlation exists between the expression levels of transcripts linked to hematopoiesis, interferon-mediated immunity, and cell trafficking and the advancement of arthritis.[27,28]

3. Application of next-generation RNA sequencing (RNA-seq) in RA research

The cutting-edge method known as "next-generation RNA sequencing" is used to analyze transcriptomes and provides extremely high yields by determining the nucleotide order in certain genomes or targeted DNA or RNA sections. The discipline of RNA sequencing is revolutionized by NGS. The three primary benefits of NGS are its cost-effectiveness, high throughput, and precise RNA characterization. The current research using RNA-seq for RA theranostics is presented in Table 2.

3.1. Novel biomarker discovery

Biomarkers are biological substances found in bodily fluids such as blood and tissues that indicate the presence of a disease. Some biomarkers for RA include rheumatoid factor, anticyclic citrullinated peptide, erythrocyte sedimentation rate, and C-reactive protein. Imaging biomarkers such as X-rays and MRIs can detect joint erosion and synovitis. Genes such as SNPs and HLA-DRB1 can also function as biomarkers, helping to determine the severity and course of a disease. Elevated levels of chemokines and cytokines during the proinflammatory phase can also be detected in certain patients with RA.[29-32]

3.2. Identification of dysregulated pathways

RNA sequencing helps in determining the RA pathways. RNA-seq facilitates the determination of transcriptome abundance levels and allows for the comparison of gene expression patterns with those of a healthy individual. The genes that are dysregulated help in indicating the molecular pathways.[23]

3.3. Gene ontology

Gene ontology helps in the processing of RNA-seq raw data. It involves quality control to remove any reading of

Table 1. Transcriptomic RNA sequencing across several conditions

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
1	Alzheimer's disease (AD)	This study successfully produced transgenic mice expressing IE2 in the hippocampal region, revealing that IE2 induces the expression of a gene related to disease-associated microglia.	PBS was administered to the mice before hippocampal tissue removal, and instructions were provided for constructing a 3′-library. Barcoded data were demultiplexed and mapped to the mouse genome.	Differentially expressed genes (DEGs) affecting the transcriptome profile were discovered in the microglia of IE2 transgenic mice, including downregulated and upregulated transcripts such as Tmem119, P2ry12, ApoE, CD83, and ISG15.	The sequencing data were analyzed using Cell Ranger or 10× Genomics Cloud Analysis through the Loupe Browser.	The study's successful development of IE2 transgenic mice presents a novel approach for investigating HCMV infection *in vitro*.	2024	17
2	Autoimmune hepatitis (AIH)	This study aimed to investigate the potential molecular pathways related to AIH and describe the characteristics of peripheral blood mononuclear cells (PBMCs) in AIH using single-cell RNA sequencing (scRNA-seq).	The study used scRNA-seq to produce single-cell transcriptomes from healthy controls and patients with AIH, revealing differential expression profiles and cell clusters to understand the biological roles of AIH immune cells.	In monocytes, the transcriptome profile showed 87 upregulated and 12 downregulated DEGs, whereas in NK cells of AIH PBMCs, the profile showed 101 upregulated and 15 downregulated DEGs.	After GO functional enrichment analysis of the DEGs, cells with similar expression patterns were grouped using the UMAP method. The GEO database was constructed using the obtained data.	AIH is an immunological condition characterized by autoantibodies, hypergammaglobulinemia, and persistent active hepatitis. Understanding the molecular mechanisms in PBMC-derived monocyte and NK cell clusters could aid AIH therapy.	2024	18
3	Diabetic kidney disease (DKD)	The researchers used CellMarker and CellPhoneDB to analyze single-cell RNA sequencing data in publicly available datasets to identify cell types and interaction networks associated with DKD.	Six DKD-related datasets were analyzed using scRNA-seq and then normalized, after which 10 genes with the highest variation were determined. DEGs of each cell subtype were detected using the Find All Markers method.	The study identified 356 DEGs as implicated transcriptomes, with 178 upregulated and 178 downregulated genes.	The regulatory relationship between genes and traits associated with DKD was evaluated using WGCNA analysis, KEGG and GO databases, Metascape and GEO databases, and other relevant information.	RNA-seq analysis revealed 356 unique DEGs in the kidney, platelet alpha granule, urogenital system, and glycosaminoglycan-binding pathways, with three immune-related genes used as DKD treatment targets and diagnostic markers.	2023	19
4	Autoimmune diseases (psoriasis and vitiligo)	This study investigated the aberrant ferroptosis- and pyroptosis-related gene patterns in autoimmune diseases such as vitiligo and psoriasis using scRNA-seq datasets and bulk RNA sequencing.	The Genome Sequence Archive (GSA) provided scRNA-seq datasets for analysis, scoring target cells based on gene expression levels in signature gene lists.	Certain pro-ferroptosis genes, including *ACSL4*, *SAT1*, *RPL8*, and *CS*, showed increased expression in the transcriptome profile.	Gene expression analysis was performed using DESeq2, sequencing was performed using Illumina HiSeq™ 2000, and scRNA-seq datasets for lesional and non-lesional skin biopsies from patients with AD were accessed through the GEO database.	The study revealed that ferroptosis and pyroptosis disrupt vitiligo and psoriasis, providing new insights into their role in the scRNA-seq-based pathophysiology of autoimmune diseases.	2022	20

(Cont'd...)

Table 1. ***(Continued)***

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
5	Breast cancer	Using RNA-seq and trajectory analysis, the researchers identified CD44+/ALDH2+/ ALDH6A1+ breast cancer stem cells (BCSCs) and verified their pluripotency.	This study involved five female patients with breast cancer, including triple-negative and non-TNBC types, and performed scRNA-seq analysis of primary tumors and matching lymph nodes.	Overall, 10 matching axillary lymphatic nodes and 96,796 individual cells from 5 primary tumors were used to construct UMAP maps. The levels of expression were represented using heatmaps.	The accession code GSE180286 was used to store the scRNA-seq data in the GEO database.	The study revealed transcriptome profiles, established the evolutionary history of carcinogenesis, described intra- and inter-tumor heterogeneity, and identified BCSCs.	2021	21
6	Systemic lupus erythematosus (SLE)	Researchers analyzed lncRNA profiles and dysregulation in the peripheral blood mononuclear cells of patients with SLE using RNA-seq of PBMCs and other published transcriptome datasets.	The study used 10 μg of total RNA. Cutadapt was used to remove adaptors and low-quality bases, and transcriptome data were retrieved from two other studies for RNA-seq data processing.	The transcriptome profiles of patients with SLE were significantly dysregulated, with >95% of coexpression combinations showing positive correlations, suggesting underlying transcription-promoting effects of lncRNAs.	Ten SLE samples were selected at random and five controls were used in the investigation. The NCBI GEO database contained the RNA-seq data.	The lncRNA pattern of patients with SLE, influenced by the Th cell differentiation-associated lncRNA AC007278.2, may regulate inflammatory response genes, improving the understanding of the role of lncRNAsin SLE etiology and development.	2021	22
7	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)or COVID-19	Whole-transcriptome sequencing of the peripheral blood samples of 14 patients with COVID-19 and four healthy donors revealed four modules in the protein-protein interaction network of upregulated and downregulated DE mRNAs.	Gene sequencing libraries were created using TruSeq Stranded RNA Total Library Prep Kit, TRIzol reagent, fastp software, miRdeep2, FPKM, miRanda, and FPKM to evaluate the accuracy of the raw data.	The transcriptome profile revealed 25,482 DE mRNAs, with 4142 upregulated and 21,340 downregulated mRNAs and 9 upregulated and 14 downregulated miRNAs, with a fold change of 2.0 and $P < 0.05$.	This study used the GenCLiP 3, GeneCards, and Online Mendelian Inheritance in Man (OMIM) databases to identify inflammation-related targets, merging increased levels of DE mRNAs and inflammation targets, resulting in an elevated expression of 149 DE mRNAs for COVID-19.	Whole-transcriptome sequencing was used to identify mRNAs, lncRNAs, and miRNAs in the peripheral blood of healthy donors and patients with COVID-19 and analyze the "cytokine storm" mechanism.	2021	23
8	Cervical cancer	The study identified 140 DEGs in cervical malignant tissues, normal tissues, and SiHa cells after transcriptome sequencing, irrespective of HPV16 E6/E7 deletion.	The study used bioinformatics to analyze 140 DEGs, revealing their primarily enriched content in the cell cycle and DNA repair, with 20 genes examined and six verified through qRT–PCR.	The study analyzed 20 genes, including *CENPI*, *KIF14*, *RACGAP1*, *FOXM*, *EXO*, *KIF4A*, *PSRC*, *C19orf57*, *MCM6*, *TLX2*, *CIT*, *NDC80*, *MCM7*, GINS2, *ORC*, *NUSAP1*, and *UHRF*.	This study utilized the TF2DNA database.	It provided a novel understanding of how HPV oncogenes maintain a malignant state at the transcriptional level.	2019	24

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Table 1. ***(Continued)***

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
9	Pancreatic ductal adenocarcinoma (PDAC)	The surrounding benign pancreatic tissues and the matching tumor transcriptomes were examined in 10 patients who underwent PDAC surgery.	RNA-seq of all 20 samples was successfully performed using nucleotide distributions and high-quality parameters, with reading sequences being 2 × 76 bp long.	The transcriptome profile revealed 2736 DEGs with a false discovery rate of <0.05, including six microRNAs and 1554 upregulated and 1182 downregulated DEGs.	The TopHat2 alignment tool mapped 95.5% of the sequenced reads to the hg19 genome assembly, with the RefSeq database providing the number of components in each identified gene.	The study used RNA-seq technology to identify a higher number of DEGs than previous microarray-based expression profiles, which can be used for biomarker testing and therapy of pancreatic cancer.	2017	25
10	Breast cancer	The study examined the transcriptome expression patterns of HER2-positive, TNBC, and non-TNBC breast tumors using Illumina technology, resulting in 1.2 billion high-quality raw reads.	Ribosomal RNA was extracted from total RNA using RiboMinus Eukaryote Kits and assembled into transcripts using Cufflinks; PCA plots were constructed and clustered using Avadis NGS software.	mRNA sequencing identified 75,000 transcripts in samples, including GNB2L1, CANX, B2M, and SPARC, affecting cell motility, antiapoptosis effect, migration, and stress management, which are linked to breast cancer progression.	The TopHat program 1.1.4 was used to align the pair-end readings. The OMIM database was used.	The mRNA sequencing–centered approach can provide a more precise deconvolution of the transcriptional and post-transcriptional aspects of breast cancer.	2012	26

Abbreviations: RNA-seq: RNA sequencing; PBS: Phosphate-buffered saline; GO: Gene Ontology; GEO: Gene Expression Omnibus; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNA: Long non-coding RNA; qRT–PCR: Quantitative reverse transcription–polymerase chain reaction; PCA: Principal component analysis.

Table 2. Recent studies on transcriptomic RNA-seq in RA

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
1	This study aimed to identify proteomic, transcriptomic, and cellular markers that indicate abatacept resistance in patients with RA.	Varying responses to abatacept in patients with RA are attributed to unknown molecular pathways. The study clarified the low effectiveness of abatacept in RA using transcriptomic RNA-seq.	Blood samples	A study involving 22 patients with RA using abatacept measured gene expression, plasma protein, and surface molecule levels using flow cytometry and RNA sequencing, with seven responders and 50 non-responders.	The researchers found that distinct monocyte-derived transcriptome features before treatment account for the differences in abatacept effectiveness. The information was obtained through transcriptomic RNA-seq.	2024	46
2	The aim of the project was to use transcriptomic RNA-seq to identify genes and pathways related to RA.	Gene expression data from 202 patients with arthritis were used to create a synovium gene expression predicting model, followed by transcriptome-wide association analysis using FUSION software and RNA-seq.	Synovium	Samples from 245 patients who underwent knee replacement surgery were subjected to transcriptomic sequencing, genotyping, RNA extraction, and RNA-seq, with the human reference genome (hg19) mapped to the findings.	Transcriptomic RNA-seq analysis using relevant synovial tissues revealed genes previously linked to RA, providing novel information on the fundamental genetic composition of RA.	2024	47
3	This study aimed to use transcriptomic RNA-seq to discover discrete populations of macrophages and their distinguishing characteristics in the RA synovium to treat RA.	In synovial cells from patients with RA, single-cell RNA sequencing or scRNA-seq was used to identify gene fingerprints and subsets of cells.	Synovium	The study visualized macrophage spatial distribution using transcriptomic and scRNA-seq data, flow cytometry, immunofluorescence, and transcription factor analysis to study the macrophage polarization markers CD86 and CD206.	ScRNA-seq identified three macrophage cell clusters in RA synovial macrophages, revealing distinct polarized states and molecular markers, helping in the development of a unique therapeutic strategy.	2023	48
4	The aim of the experiment was to discover novel targets for therapy and provide fresh insights into the peripheral blood immunological processes of RA using transcriptomic scRNA-seq.	The study used scRNA-seq data from four RA samples and single-cell transcriptomic data from healthy control samples to understand RA development mechanisms, identify therapeutic targets, and develop disease settings.	Peripheral blood samples	Single-cell 3′-gene level libraries were generated from peripheral blood samples and processed using Cell Ranger software, and DEGs were identified using the Find Markers function of the Seurat package.	This study described the present status of the immune system and cell communication in the peripheral blood of patients with RA, including the gene expression patterns, PBMC abundance, and alterations in signaling pathways. Furthermore, it discovered a number of important cell subpopulations and particular genes that aided in the discovery of novel therapeutic targets.	2023	49

(Cont'd...)

Table 2. ***(Continued)***

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
5	Transcriptomic RNA-seq was used in this study to address RA remission mechanisms, provide predictive biomarkers, and gain a deeper understanding of the role played by distinct fibroblast populations in the RA process.	To identify HBEGF+ fibroblasts and ascertain the number of HBEGF+ fibroblasts in various joint conditions (health, K/BxN serum transfer arthritis (STA), and STA remission), the study used two single-cell RNA sequencing datasets of mouse synovial cells.	Synovium	Thirteen datasets, including RNA-seq and single-cell transcriptomics, were used to analyze synovial tissue in 102 patients with arthritis, comparing gene expression between HBEGF+ and HBEGF− fibroblasts.	Fibroblasts in the synovial fluid are believed to play a crucial role in controlling joint homeostasis in RA. Using transcriptomic RNA-seq, the study found that HBEGF+ fibroblasts contribute to RA remission and that HBEGF may be a novel biomarker for predicting RA progression.	2022	50
6	To ascertain whether gene–gene interactions in a network analysis of synovial samples obtained during the early phases of RA using RNA-seq could contribute to our understanding of the pathophysiology of RA and improve treatment response in prediction models.	The study was designed to collect 10,537 experimentally confirmed gene–gene interactions using four carefully selected route libraries. After characterizing histologically defined pathotypes in early RA using synovial RNA-seq, we extracted particular gene–gene interaction networks and utilized these synovial-related gene–gene networks to predict the response to methotrexate-based disease-modifying antirheumatic drug (DMARD) therapy. Next, by statistically evaluating each network with robust linear regression models, the study revealed the differential interactions within each network.	Synovium	The study examined RNA-seq data from 94 patients with RA who started methotrexate-based csDMARD therapy after 6 months, evaluating gene–gene interactions through rigorous regression analysis.	To improve models for predicting treatment response in RA, the study included a unique, potent method known as transcripts micRNA-seq, which leverages physiologically significant gene–gene interactions through gene interaction networks.	2022	51
7	The aim of this study was to gain a complete understanding of the patterns of expression across the genome in synovial tissue samples from patients with RA to identify potential mechanisms regulating the onset and progression of RA.	RNA-seq was used to determine the transcriptomic patterns of synovial tissue specimens from nine patients with RA who were members of the East Asian community. All identified genes were examined using gene set enrichment analysis (GSEA), and DE-seq was used to identify differentially expressed genes (DEGs). Quantitative real-time PCR (qRT–PCR) was used to verify the most important hub genes.	Synovium	Samples of synovial tissue were collected from nine patients with RA. Total RNA was then extracted from the synovial tissue. Total RNA samples with RIN > 7.0 and 28S/18S ≥ 0.7 were subjected to RNA-seq. Then, libraries were constructed using TruSeq Stranded mRNA LT Sample Prep Kit. The Illumina HiSeq ×10 platform was used for assembling the libraries. The accuracy of the RNA-seq technique in detecting DEGs was evaluated, and the expression levels of the 10 identified hub genes were quantified using qRT–PCR.	This study identified and validated DEGs in synovial tissue samples from patients with RA using transcriptomic RNA-seq. It also highlighted the activity of a subset of chemokine genes and provided novel insights into the molecular mechanisms of RA pathogenesis. Finally, it identified potential targets for screening and treatment.	2022	52

(Cont'd...)

Table 2. ***(Continued)***

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
8	The most prevalent internal alteration found in eukaryotic mRNAs is N6-methyladenosine (m6A), and it exerts a direct effect on the development and course of several diseases, particularly tumors. This study investigated the relationship between m6A methylation and RA using transcriptomic RNA-seq.	The aim of this study was to identify different amounts of m6A methylation in human RA using two high-throughput sequencing techniques.	Synovium	The study used the MH7A cell line to detect m6A methylation in RA, confirming the expression levels in MH7A cells and synovial tissues of AA rats.	A total of 206 genes with differentially regulated m6A methylation were detected using m6A-seq, of which 118 had substantially elevated m6A methylation and 88 had significantly decreased m6A methylation. In MH7A cells, the transcriptional mapping of m6A was performed, and a possible correlation between the RNA methylation alteration and RA-related genes was noted.	2021	53

Abbreviations: RNA-seq: RNA sequencing; RA: Rheumatoid arthritis.

poor quality. To identify the differentially expressed genes (DEGs) based on fold change and statistical significance criteria, techniques such as DEseq2 and limma-voom are used. Moreover, it performs DEG annotation using databases and bioinformatics tools, and DEG maps are used to identify pathways that are enriched in dysregulated genes according to predetermined criteria. Bar plots, bubble plots, and network diagrams are useful for showing the most important pathways.[33,34] Dysregulated pathways in RA include T-cell receptor signaling, altered B-cell receptor signaling, autoantibodies, and the Toll-like receptor. Type I IFN signaling is activated in RA, contributing to inflammation and joint destruction. The signal transducer and activator of transcription (STAT) regulates inflammation and the immune response, whereas nuclear factor-κB regulates gene expression. Abnormal expression of the Janus kinase–STAT pathway and the mitogen-activated protein kinase (MAPK) signal transduction pathway also contribute to RA pathogenesis.

3.4. Personalized medicine

The traditional treatment for RA involves medication, psychotherapy, and rehabilitation to maintain the locomotor system and reduce discomfort, inflammation, and osteoporosis. Weak opioids, muscle relaxants, and analgesics are used to manage pain, whereas strong opioids such as morphine are considered in certain cases. RNA-seq profiling can predict prognosis, treatment response, and disease progression. Synthetic disease-modifying antirheumatic medications include cyclosporine A, leflunomide, azathioprine, methotrexate (MTX), sulfasalazine (SSZ), and cyclophosphamide. RA is diverse, with MTX being the first-line treatment in 40 – 60% of cases.[35,36]

3.5. Immune dysregulation

Analyzing gene expression in immune cells and the synovium can assist in identifying dysregulated pathways and RA etiology using RNA sequencing. The expression patterns of cytokines and chemokines in the synovium and other tissues of patients with RA were also examined. Tumor necrosis factor-alpha, interleukin-6, and other chemokines can be used to identify the pathophysiology of RA. RNA-seq enables the profiling of autoantigens, immunological tolerance, and autoantibodies. Autoimmune problems are significant for patients with RA. Combining all omics data helps in the study of immune dysregulation (genomics and proteomics).[37-39]

4. Transcriptomic profiling of synovial tissue and fluid in patients with RA

The synovium, a thin tissue layer, aids in the function of diarthrodial joints by lining the fibrous capsule.[40] In healthy joints, it generates lubricin and hyaluronate for synovial fluid, supporting flexion and load-bearing without compromising bone and cartilage integrity. The inflammatory synovium in RA exhibits tissue hyperplasia, angiogenesis, and leukocyte infiltration, with fibroblasts playing a vital role in cartilage destruction, leukocyte influx and outflow, and bone damage.[41]

In RA, proinflammatory chemicals such as IL-6 and CCL5 are generated, synovium fibroblasts are constantly active, and matrix remodeling enzymes such

as matrix metalloproteinase (MMP)-9 and MMP-3 are secreted. Moreover, enhanced proliferation and reduced responsiveness to apoptotic signals are observed. Consequently, the synovium develops an aggressive nature, resulting in inflammation and hyperplasia, ultimately causing discomfort and loss of function. However, over the past 10 years, several fibroblast subpopulations have been identified through transcriptomic technologies and single-cell sequencing.

In each previously reported experiment, the synovial tissue was collected from limbs that had been amputated or from victims' limbs within 6 h of their death. Studies often end at one of two resolution levels: whole-tissue samples or individual cellular populations.[42,43] Two primary methods are commonly used for the isolation and investigation of synovial cells, also known as synovial fibroblasts: the explant outgrowth approach and the enzymatic digestion approach. The enzymatic digestion strategy focuses on breaking down the extracellular matrix and disrupting cell adhesion to this matrix to generate a suspension of synthetic cells.[43] However, a range of enzymes, concentrations, and digestion durations can be used to achieve this. Owing to the straightforward nature of the explant outgrowth approach, it is reported more frequently. When small segments of synovial tissue are cultivated, adherent cells can move out of the tissue and begin to proliferate.[42,44] After 7 days, the remaining tissues should be removed to cultivate cells. Although the explant outgrowth approach is easier to use than the enzymatic digestion approach, it has certain limitations. Favoring synovial populations that proliferate rapidly and are mobile enough to escape the tissue while ignoring other populations is risky. Furthermore, there exists a technique for cryopreserving the synovial tissue that minimizes batch effects and permits further digestion.[45] To avoid any contaminated proteins or DNA, the extracted RNA is purified. Reverse transcriptase is used to convert RNA into cDNA. Polymerase chain reaction (PCR) is used to amplify the fragmented cDNA library to provide sufficient material for sequencing. An Illumina NovaSeq or HiSeq sequencing platform is used to load the prepared library.

Low-quality reads and adaptor sequences are eliminated from the raw sequencing reads through processing. The abundance of each transcript is measured using bioinformatics techniques (gene expression analysis). Functional analysis of the DEGs is performed to gain insights into their biological functions and possible roles in the pathophysiology of RA. Researchers can use RNA-seq to obtain useful information on the gene expression profile of synovial cells in RA by following the techniques depicted in Figure 3. Using these data, we can better understand the mechanisms underlying RA and establish new diagnostic and treatment targets.

5. Identification of DEGs associated with RA pathogenesis

The most common type of autoimmune inflammatory arthritis in adults is RA. In addition to increasing the mortality rate, RA significantly reduces the quality of life in terms of health and impairs one's ability to perform everyday chores, including domestic and job-related responsibilities.[37,41] Five of every 1000 people have RA, which certainly causes serious damage to joints and disability.[54] Joint synovitis, which can appear as exudation, synovitis, or other diseases in the joint cavity, is a

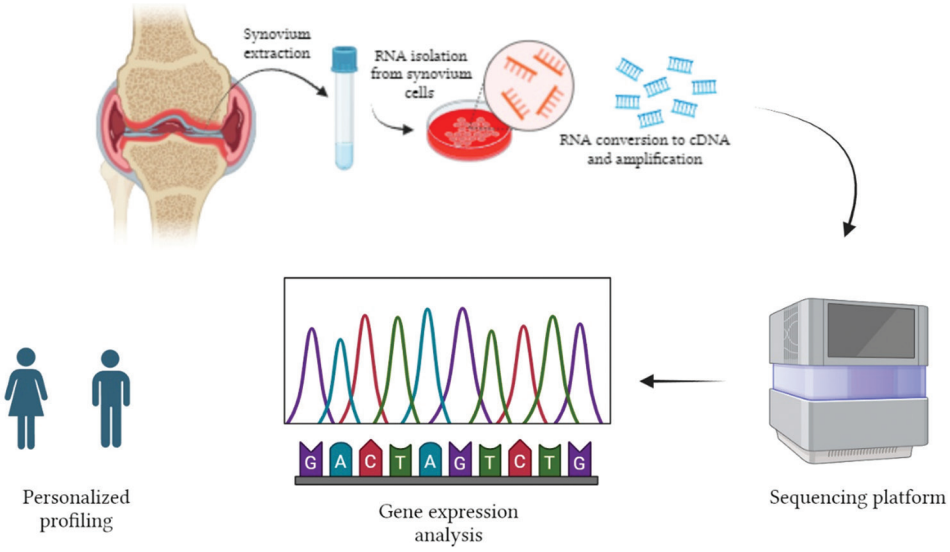


Figure 3. Transcriptomic gene expression profiling of synovial cells in rheumatoid arthritis

pathogenic feature. Finally, these disorders result in bone and cartilage dysfunction as well as stiff joints.[55,56] RA is a severe inflammatory autoimmune disease that is affected by both hereditary and environmental factors. In recent years, new genes and the close relationship between genetic and epigenetic processes have received considerable attention.[57] Serological testing (rheumatoid factor and anticyclic citrullinated peptide) and magnetic resonance imaging are the most frequently used techniques for the early detection and diagnosis of RA.[58] Nevertheless, these methods have limitations, and therefore, the precision and accuracy are poor.[59] X-rays and computed tomography can only identify lesions that are advanced in the stage; they cannot identify early damage. At present, there is no established therapy for RA, and its origin remains unknown. An increasing number of studies indicate that it is associated with genetics, immunological problems, endocrine variables, environment, and other factors, resulting in long-term inflammatory joint lesions. The recurrence and continuation of numerous complicated diseases or biological processes are intimately linked to the DNA methylation control of gene expression.[60] DNA methylation is crucial for preserving the expression profile unique to a given tissue, blocking the expression of foreign and viral genes, imprinting gene expression, and controlling chromatin stability.[61] DNA hypermethylation often prevents transcription factors from binding to the target area, which can restrict gene production; however, some studies showed that hypermethylation can also increase gene expression. The findings still require further investigation because of their inconsistency. Moreover, a plethora of research indicates that DNA methylation could play a role in the onset and progression of RA.[62] Gene expression microarrays have primarily been used to examine gene expression profiles, which provides a reasonably novel approach to exploring genes and numerous opportunities for drug-based molecular targeting applications. Numerous investigations of RA on methylation or expression microarray data profiling have been conducted in recent years, and hundreds of differentially methylated and differentially expressed genes (DMGs) have been identified.[63-67]

6. Regulatory networks and signaling pathways revealed by RNA-seq in RA

RA and osteoarthritis are joint diseases influenced by genetics, nutrition, and environmental factors. Transcriptomics and RNA sequencing can help differentiate between the two by examining gene expression.[51]

6.1. Role of RNA-seq in RA research

RNA-seq is a crucial technique in the study of RA, providing insights into the transcriptome and molecular mechanisms. It identifies genes that are upregulated or downregulated, allowing researchers to evaluate gene expression levels and identify coding and non-coding RNAs linked to RA. This information can be used for prognosis, therapy response monitoring, and disease diagnosis. RNA-seq also profiles gene expression in specific cell types, helping in targeted therapeutic development and drug discovery.[51,68,69]

6.2. Dysregulated gene expression in RA

RA is caused by dysregulated gene expression, resulting in inflammatory and autoimmune processes that damage joints. Dysregulated genes produce proinflammatory cytokines, causing synovial inflammation, cartilage degradation, and bone erosion. Autoantibodies and immune complexes cause tissue damage and synovial inflammation. Dysregulated transcription factors and abnormal signaling networks promote inflammation and immune cell activation. Anti-inflammatory mediators such as TIMPs and IL-10 balance proinflammatory and anti-inflammatory responses. Identifying these genes is vital for developing customized therapies to improve the prognosis of patients with RA.[4,70,71]

6.3. Regulatory networks in RA

Key transcription factors, including NF-κB, AP-1, and STAT3, exhibit dysregulated activity in RA, as shown by RNA-seq.[72,73] In the synovium of patients with RA, these factors are critical in regulating the production of proinflammatory cytokines, chemokines, and other inflammatory mediators. Gene dysregulation in RA is affected by epigenetic modifications, including chromatin structural alterations, DNA methylation, and histone acetylation.

Non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have also been shown to play a role in RA pathophysiology. These RNAs possess the ability to target genes related to immunological responses, inflammation, and bone/cartilage homeostasis. In cRNAs interact with chromatin-modifying complexes or control the expression of target genes related to inflammatory signaling pathways and immunological responses. Moreover, circular RNAs act as microRNA sponges, absorbing miRNAs and altering their activity to indirectly regulate gene expression in RA-related pathways.[72,73]

6.4. Signaling pathways implicated in RA

Numerous signal transduction pathways affect the course of RA, and aberrant signals are frequently used as targets for developing medications. One of the most important members of the JAK family, the JAK-STAT pathway, controls apoptosis, cell differentiation, proliferation, inflammation,

and immunological function. The pathological foundation of RA is synovitis, and a variety of inflammatory responses have been observed in the synoviums of patients with RA (the soft tissue lining the interior of joints, tendon sheaths, and bursae is known as the synovium, also known as the synovial membrane or stratum synoviale). By suppressing JAK2 and STAT1, RA can be treated by activating T and B cells and preventing the production of cytokines.[74]

The pathogenic process of RA is intimately associated with the MAPK signaling system. The most significant member of the MAPK family, P38 MAPK, is activated in endogenous immune cells and can thicken the synovium, activate transcription factors, and significantly increase the production of inflammatory chemokines. It may also contribute to T-cell differentiation and prevent cell death. Angiogenesis, proliferation, metabolism, and cell survival are all regulated by the PI3K-AKT pathway, an intracellular mechanism that has been associated with the onset and progression of RA. The pathophysiology of RA is aided by the abnormal activation of this pathway, which increases the production of inflammatory chemicals and cytokines.[75]

In the PI3K/AKT/mTOR pathway, the mammalian target of rapamycin (mTOR) suppresses autophagy, encourages aberrant synovial cell proliferation, and is essential for osteoclast survival and differentiation, all of which exacerbate RA. SYK and BTK are two important molecules involved in B-cell receptor signaling, whose dysregulation has been linked to the etiology of RA. Phosphorylated SYK and BTK are expressed at higher levels in peripheral B cells in patients with RA, which is associated with the formation of autoantibodies. Regarding the treatment for autoimmune conditions such as RA, BTK is a desirable target.[75]

By controlling bone metabolism and synovial inflammation, the Wnt signaling pathway is essential in the pathophysiology of RA. Studies have demonstrated that the inhibition of NAV2 expression stops the development of RA and reverses phenotypes associated with inflammation, making it a prospective target for RA therapy.[74,75]

7. Integration of multiomics data to clarify the molecular mechanisms underlying RA

The term "multiomics data" describes the synthesis of several types of molecular data from different omics technologies, including transcriptomics, proteomics, metabolomics, and epigenomics.[76-78] Every omics technique provides a limited perspective on the various facets of biological systems. Proteomics investigates proteins and their alterations, metabolomics explores small molecule metabolites, transcriptomics examines gene expression levels, and epigenomics investigates epigenetic changes that control gene expression. Genomics is primarily concerned with DNA sequences and genetic variants. Combining these disparate omics datasets can enable researchers to obtain a more detailed knowledge of intricate biological processes and illnesses such as RA. A more comprehensive understanding of the molecular mechanisms underlying the pathophysiology of RA is possible through integration, which enables the investigation of interactions and correlations between genes, proteins, metabolites, and epigenetic variables.[79,80] RA is a mixed disease involving numerous biological processes, including inflammation dysregulation and tissue damage. Multiomics approaches allow an in-depth overview of the molecular landscape by continuously analyzing multiple biological layers of bio information such as transcriptomics and genomics. RA exhibits a diverse clinical presentation and response to treatment. Mutiomics data help identify molecular subtypes or clusters within patients, which helps in the treatment of particular individuals. Multiomics data help interconnect molecular pathways and networks and identify signaling pathways driving disease progression. Multiomics helps discover and develop new therapeutic agents for patients with RA. Researchers can repurpose current medications with established effects on relevant molecular pathways and prioritize prospective targets for therapeutic development by identifying molecular targets and pathways implicated in the disease etiology.[81,82]

8. Benefits, limitations, and significant advances of the technique

Several simple and complex diseases are poorly understood; hence, the analysis of their transcriptome is a vital method to investigate various diseases such as RA. Next-generation RNA-seq is a high-throughput method for the characterization of gene expression levels. Therefore, the use of RNA-seq in RA research has several benefits. This method can detect all RNA transcripts irrespective of the genes known or presently unknown, which provides a better view of the gene changes occurring in RA. RNA-seq provides precise and quantitative readouts of gene expression, therefore making it possible to capture differences in gene expression kinetics. It can also capture cases of alternative splicing, which results in the production of different proteins that are functionally different. RNA-seq can also identify unidentified RNA forms, for instance, ln cRNA and miRNA, which are responsible for the regulation of gene expressions. RNA-seq data obtained from RA samples can be combined with other omics data, including genomics and proteomics data, to further investigate the pathogenesis of RA.

Nevertheless, at the same time, there are some limitations to using RNA-seq. For instance, when performing large-

scale studies, RNA-seq can be relatively expensive. The processing of RNA-seq data is a delicate process and thus requires the use of specific visualization tools as well as extensive knowledge in the field of molecular and cell biology. Other limitations include specimen factors such as RNA degradation and sequencing errors. As mentioned earlier, RA is a heterogeneous disease and RNA-seq may not be an ideal method to detect upfold changes of genes in different cellularity of the joint.

RNA-seq has led to significant advances in our understanding of RA pathogenesis. It has provided novel genes of RA that are involved in immune cell activation, inflammation, and destruction of joints. RNA-seq has played some roles in the function of immune cells involved in RA, including Th17 cells, different T regulatory cells, and macrophages. It has provided tremendous insights into novel regulatory RNA molecules, including lncRNAs and miRNAs involved in the pathogenesis of RA. Some of the genes involved in immune cell signaling and inflammation have been proposed as therapeutic targets for RA using RNA-seq technology.

9. Conclusion

The use of next-generation RNA-seq has completely changed our knowledge of the intricate transcriptome landscape associated with RA. This review examined the use of RNA-seq in analyzing the pathophysiology of RA, ranging from defining dysregulated pathways and gene expression patterns to discovering novel biomarkers. Important discoveries include the identification of alternative splicing events, DEGs linked to RA pathophysiology, and the potential use of lncRNAs and circular RNAs as diagnostic tools. The complex regulatory networks and signaling pathways involved in RA have also been clarified using RNA-seq. Researchers can obtain a more comprehensive understanding of the molecular pathways causing RA by combining multiomics data. These findings are extremely promising for the development of tailored treatment approaches, improvement of diagnostic capabilities, and eventually, the discovery of novel therapeutic targets for individuals with RA. Prospective avenues for investigation may focus on overcoming constraints such as data analysis obstacles and integrating spatial transcriptomics data to comprehend cellular heterogeneity in the combined microenvironment. Certainly, additional research into the potential of RNA-seq will result in important discoveries in the field of RA research, providing better patient outcomes.

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