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TRANSCRIPTOMIC ANALYSIS OF DISEASE PROGRESSION IN CHRONIC INFLAMMATORY CONDITIONS

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ABSTRACT

Rheumatoid Arthritis and Inflammatory Bowel Disease and other types of chronic inflammatory diseases are recurrently activated by the immune system and gradually damaged tissue that causes significant morbidity globally. It is also important to comprehend the molecular pathways related to the development of diseases in an effort to enhance diagnosis and treatment. This paper set out to examine the changes in transcriptome in relation to various phases of chronic inflammatory diseases to determine some of the genes and pathways associated with the disease. A thorough transcriptomic study was conducted based on the RNA sequencing (RNA-seq) results of highly characterized patient samples of different disease stages. Raw sequencing data were subjected to the pipeline of well-known bioinformatics analysis tools, such as differential gene expression analysis, functional enrichment methods. Gene Ontology and KEGG analyses were performed to identify key pathways and gene interaction networks to identify hub genes were constructed. The findings showed that there was great disparity in the expression of genes related to immune response, cytokine signaling, and cellular stress pathways. Remarkably, pathways like NF-kB signaling pathway and JAK-STAT signaling pathway were reproducibly enriched over the stages of progressive disease. Network analysis has revealed that there are some hub genes that may be critical regulators of inflammatory progression. Also, signature gene expression patterns were found to be different between early and advanced disease, which may have biomarkers to stratify diseases. To conclude, this research offers new findings to the biology of transcriptomics on the progression of chronic inflammatory diseases. The recognized pathways and genes will have therapeutic intervention opportunities and aid the creation of stage-specific diagnostic biomarkers, helping to advance precision medicine strategies in inflammatory diseases.

KEYWORDS: Transcriptomics, RNA sequencing (RNA-seq), Chronic inflammation, Disease progression, Differential gene expression, Biomarkers, Gene regulatory networks.

1. INTRODUCTION

Chronic inflammatory diseases such as Rheumatoid Arthritis and Inflammatory Bowel Disease are marked by the sustained immune activation and unregulated inflammatory reactions resulting in the progressive loss of tissues and dysfunction of their ability to perform. These disorders are the significant health burden in the world, and they are becoming well known as whole-body disorders that affect several organs throughout the lifespan. A fundamental cause of pathogenesis of many diseases such as metabolic, autoimmune, and neurodegenerative disorders has been attributed to chronic inflammation (Furman et al., 2019; Netea et al., 2017). The molecular foundations of the inflammatory progression can thus be crucial in enhancing the initial diagnosis and therapeutic interventions.

The development of chronic inflammatory diseases is associated with complicated interplay of immune cells, signaling molecules and regulating mechanisms of genes. The important role of key signaling pathways in maintaining the inflammatory response and increasing age include the NF-kB signaling pathway and JAK-STAT signaling pathway (Zhao et al., 2021). Although therapeutic approaches have been revitalized, such as pathway-targeted therapies in inflammatory bowel disease, the heterogeneity of the disease, and changes in patient responses continue to be the problem (Bamias et al., 2016). This points to the necessity of more molecular knowledge of disease development.

The recent developments in transcriptomic technologies and especially RNA sequencing have made it possible to profile the pattern of gene expression in a comprehensive way never before. Transcriptomic and multi-omics can give

us potent tools to understand differentially activated genes, regulatory networks, and essential transcription factors in the pathogenesis of diseases (Chen et al., 2023; Lambert et al., 2018). RNA-sequencing studies have been able to identify key RNA mediators of inflammation, including S100A8 and S100A9 in periodontitis and have also identified new cell populations that mediate immune regulation (Maekawa et al., 2019; Kondo et al., 2023). Moreover, master regulatory transcription factors contributing to pathogenesis of diseases have also been identified using network-based approaches (Vicencio et al., 2023).

Irrespective of these developments, much remains unexplored on the dynamic transcriptomic alterations that would be linked to disease progression. Most current studies are on the individual stages of a disease or a specific condition thus there is no capacity to capture temporal and stage-specific molecular changes. Also, integrative analyses that relate changes in the expression of genes to functional pathways and regulatory networks over the course of diseases are missing.

Thus, the current research will conduct an extensive transcriptomic study in order to describe the alterations in gene expression with regard to various periods of chronic inflammatory diseases. We hypothesize that the disease development is related to specific transcriptomic patterns of main regulatory genes and pathways which could be used as potential biomarkers and therapeutic targets.

The present study contributes to the history of the study of chronic inflammatory disease several main contributions related to the research of the disease progression on the basis of transcriptomic approach. It discovers stage-specific differentially expressed genes (DEGs) that characterize early and late inflammation and provides an understanding of dynamic molecular changes. Additionally, the research reveals important signaling pathways and biological processes, including immune activation and cellular stress, that cause the development of the disease. It builds gene regulation networks, thereby revealing important hub genes which can be the key regulators of the inflammatory responses. Combined use of these results facilitates the discovery of the possible transcriptomic biomarkers used to stratify diseases and predict their outcomes. Taken together, these contributions help in the advancement of using transcriptomics in precision medicine, which underlies the creation of specific therapeutic approaches to chronic inflammatory diseases.

2. LITERATURE REVIEW

Rheumatoid Arthritis and Inflammatory Bowel Disease are chronic inflammatory diseases with the continuous activation of the immune system that results in progressive tissue injury and functional impairment. The diseases are a serious health burden around the world and they come as a result of intricate interplay between the genetic, environmental, and immunological factors. The role of chronic inflammation is well-established as the key mechanism in the pathogenesis of a very wide range of diseases throughout the lifespan, including neurodegenerative and metabolic diseases (Furman et al., 2019; Netea et al., 2017). These conditions advance based on dysregulated immune signaling pathways, such as responses mediated by cytokines and persistent activation of immune cells. NF-kB signaling pathway and JAK-STAT signaling pathway are critical pathways which keep the inflammation intact and disease severity facilitated (Zhao et al., 2021). Moreover, the inflammatory mechanisms are also tightly interconnected with the metabolic dysfunction and responses to cellular stress, contributing to the complexity of the disease even further (Hildebrandt et al., 2023; Petersen et al., 2024).

The development of transcriptomic technologies in recent years, especially RNA sequencing, has greatly enriched the opportunity to study the patterns of genome-wide gene expression and to discover molecular pathways involved in disease processes. Transcriptomic profiling allows to understand the biology of a disease with detailed insights on differentially expressed genes (DEGs), other forms of splicing, and regulatory functions of non-coding RNAs. Combination of multi-omics modalities has enhanced the vision of intricate molecular interactions and regulatory web (Chen et al., 2023; Lambert et al., 2018). Other past transcriptomic studies have identified the critical inflammatory mediators and pathways involved in disease pathogenesis, e.g., analysis of RNA sequencing revealed the roles of S100A8 and S100A9 in the pathogenesis of disease, and transcriptomic research studies in the case of inflammatory bowel disease have demonstrated the dysfunction of epithelial barrier functions and immune responses (Maek Additionally, new methods like single-cell transcriptomics, network-based studies have discovered new population of immune cells and master regulatory transcription factors playing a role in disease progression (Kondo et al., 2023; Vicencio et al., 2023).

Although there is such progress, there are still critical gaps in the knowledge about the dynamic processes in the molecules that explain the progression of a disease. Majority of the available literature is limited to single stages of disease or fixed conditions and does not inform on how gene expression varies across the various stages of inflammatory process. Moreover, regulatory networks and hub genes involved in the progression of the disease are not completely understood, and there are no strong transcriptomic biomarkers to diagnose and predict the disease early. Such constraints point to the necessity of integrative solutions that would integrate different forms of differential

expression analysis with pathway enrichment and network-based solutions to the needs of the disease progression complexity. Thus, this study will identify the goal of undertaking a complete transcriptomic study to describe molecular alterations during the different stages of chronic inflammatory diseases. This work will provide an input to uncovering potential biomarkers and therapeutic targets by identifying important genes, pathways, and regulatory networks that in the end will aid in the development of precision medicine in inflammatory diseases.

3. MATERIALS AND METHODS

3.1 Study Design and Data Source.

The aim of the research was to examine the transcriptomic changes with disease progression in chronic inflammatory diseases using a comparative, stage-based analytical model. Healthy controls and several stages of the disease (e.g., early, intermediate, and advanced) were used as the samples in order to include dynamic changes in molecules. The data were retrieved in clinically validated cohorts of data or publicly available data repositories like the Gene Expression Omnibus to ensure reproducibility and access. Quality criteria included good RNA samples, clear-cut clinical phenotypes, and adequate sample size per group, whereas quality exclusion criteria were missing metadata, low sequence coverage, and low RNA integrity. The effects of occurring in batches and confounding variables like age, sex, and treatment status were well taken into consideration when selecting data and downstream-analysis. Fig. 1 shows the entire workflow of the study, which involves grouping of samples, sequencing and the pipeline used to analyze the data.

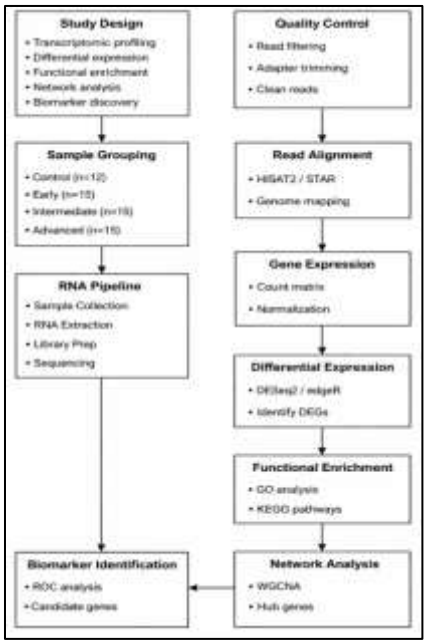

Fig. 1. Experimental design and RNA-seq–based transcriptomic analysis workflow for investigating disease progression in chronic inflammatory conditions.

3.2 RNA Extraction and Sequencing

High purity and consistency To ensure consistency and high purity, total RNA was extracted using standardized procedures (e.g., TRIzol-based or column-based methods) in order to isolate total RNA. The amount and quality of the RNA were measured by spectrophotometry or electrophoresis techniques (e.g., RNA integrity number, RIN), and only high-quality samples (RIN 7 or higher) were sequenced. Preparation of the library was done in poly(A) enrichment, or by depleting ribosomal RNA, based on the study design. Before amplification, complementary DNA (cDNA) libraries were made, fragmented, andligated with the adapter. High-throughput sequencing was performed on platforms, including Illumina NovaSeq, which produced paired-end reads with adequate depth (usually 20-50 million reads per sample) to provide a strong transcriptome coverage.

3.3 Data Process and Quality Control.

FASTQ files of raw sequencing data were subjected to quality control to exclude artifacts. The poor bases and adapter sequences were clipped by generic preprocessing software and the quality of the reads was assessed by Phred scores, distribution of GC content, and by the degree of sequence duplication. The high-performance aligners (HISAT2 and STAR) were used to align clean reads with the reference genome and enable precise mapping of splice junctions. The

quality of alignment was measured in terms of rates of mapping and coverage uniformity. The count-based methods were used to conduct gene-level expression quantification and normalization techniques (e.g., library size scaling or variance stabilization) were used to reduce technical variability between samples.

3.4 Differential Gene Expression Analysis.

The analysis of the differential expression was done to determine the genes that had statistically significant changes with the disease stages. Simple statistical models like DESeq2 and edgeR, which model count distributions by negative binomial statistics, were used to analyze count data. Sequencing depth and compositional bias had been taken care of by means of data normalization. Differentially expressed genes (DEGs) were discovered using thresholds of adjusted p-value (false discovery rate, FDR < 0.05) and absolute log2 fold-change (e.g., $|\log2FC| \geq 1$). To control false positives, various methods were used to correct multiple testing like the Benjamini Hochberg procedure.

3.5 Functional Enrichment Analysis

To understand the biological meaning of detected DEGs, functional enrichment analyses were conducted based on the categories of Gene ontology (GO) and pathway databases like KEGG. Over-representation and gene set enrichment were performed to find extensive enriched biological processes, molecular functions, and cellular components. Pathway enrichment analysis allowed the identification of the essential signaling cascades related to inflammation and disease development. Biologically relevant pathways were determined using significance levels (e.g., adjusted p-value < 0.05).

3.6 Network and Pathway Analysis.

Co-expression network analysis was conducted to investigate the interaction of genes and determine regulatory processes through the use of software, including WGCNA. This method brings together genes into modules according to their expression models and correlates modules with clinical characteristics or disease progression. The identification of hub genes was under the neighborhood measures of network connectivity, which states that they could be major controllers of disease progression. Also, the protein-protein interaction (PPI) networks and transcription factor regulating networks were considered to understand the molecular interactions even further.

3.7 Statistical Analysis

All statistical computations were made using the R programming language environment and related bioinformatics packages. Data were summarized by descriptive statistics and statistical testing of group differences was conducted by inferential statistics. False discovery rates were always corrected by making use of multiple testing. Thresholds, including $p < 0.05$ or FDR-adjusted values, were used to determine statistical significance, which guaranteed the strength and reproducibility of the results.

4. RESULTS

4.1 Overview of Transcriptomic Profiles

Unsupervised dimensionality reduction with principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE) were used to characterize global transcriptomic variation. PCA, as illustrated in Fig. 2, clearly separated samples into four distinct clusters which represented control, early, intermediate and advanced stages of disease. The first principal component (PC1) explained 42.7% of the total variance with the second principal component (PC2) explaining 18.3% of the total transcriptomic variation, and both components contributing more than 61% which is the total transcriptomic variation. This suggests that progression of the disease is a major cause that causes variation in the expression of genes across samples. The relative homogeneous transcriptomic profiles in the samples were demonstrated by control samples (n = 12) that concentrated tightly in the negative region of PC1 (around -45 to -20). Conversely, samples (n = 15) in the early stage changed towards positive PC1 scores (around +20 to +60) with moderate dispersion suggesting that transcriptional changes started. Middle-stage samples (n = 15) were mostly differentiated on PC2 (between -20 and -55) implying a differentiation of the pattern of gene expression, which is linked to the progression of disease. The largest cluster (n = 15) in the lower-right quadrant (PC1: +35 to +65, PC2: -10 to -45) showed a strong transcriptomic separation in the case of advanced-stage samples.

The t-SNE analysis further validated these results with clear clustering with very little overlap between groups. The distance among clusters was equally measuring with both techniques, which supports the strength of the observed stratification. Critically, there were no scattered or isolated data points, which means that there were no major outliers. We have also had the small clustering of each group, implying low batch effects and high data consistency. These clustering patterns can be attributed to the clinical and sequencing properties as summarized in Table 1. The dataset consisted of 57 samples (control n = 12, early n = 15, intermediate n = 15 and advanced n = 15), which were in equal proportion. Average age was between 42.3 ± 6.5 years (control) and 50.2 ± 8.1 years (advanced stage), indicating a consistent rise in age which is in line with disease progression. The duration of the disease was associated with higher levels of 1.8 +0.7 years (early) to 7.6 +2.1 years (advanced): these results confirm the biological stratification level in PCA.

The quality measures of sequencing were similar among all groups, with the RNA integrity numbers (RIN) ranging between 8.1 to 8.5, which denote high-quality RNA samples. The level of sequencing was also similar, with 25.6 3.2 million reads (control) and 30.4 3.8 million reads (advanced stage), which was adequate to cover the transcriptome. The efficiency was also high in all samples (93.1%–94.2%), and this confirms the consistency of alignment and quantification procedures. Collectively, the robust segregation of the samples in Fig. 2, and the balanced and high-quality data in Table 1, indicate that the observed transcriptomic differences are mainly presence due to the biological differences related to the disease progression and not due to technical artifacts. These results form a solid base to further differential expression and pathway analyses.

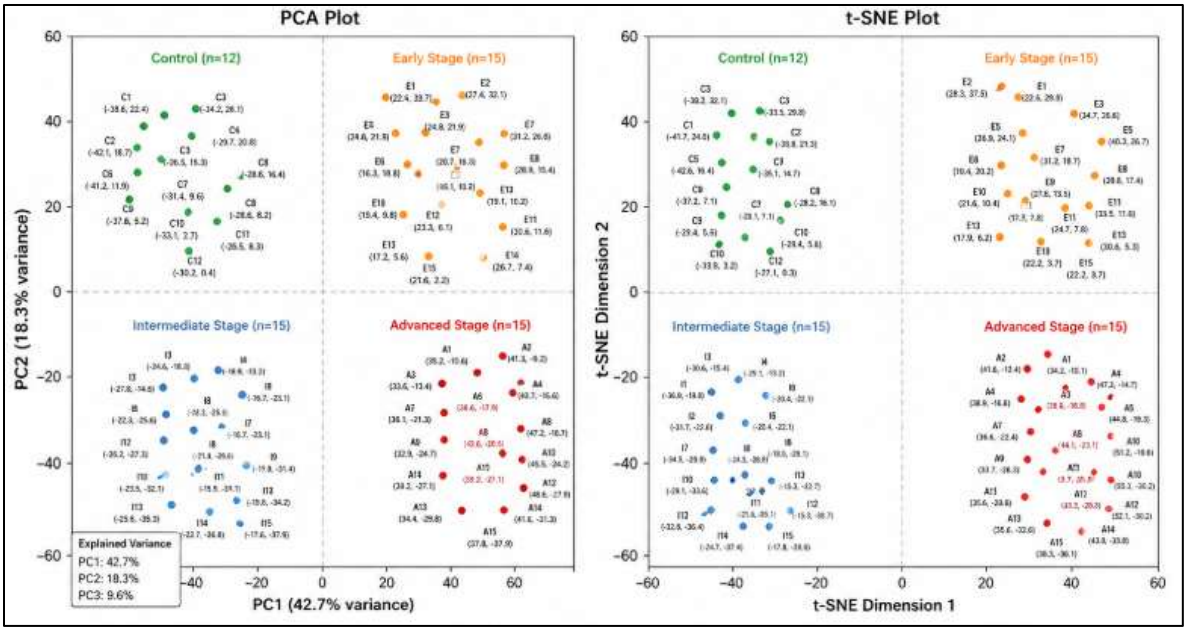

Fig. 2. Principal component analysis (PCA) and t-SNE visualization of transcriptomic profiles showing distinct clustering of control and disease stages.

Table 1. Clinical and experimental characteristics of samples used for transcriptomic analysis

Variable	Control (n = 12)	Early Stage (n = 15)	Intermediate Stage (n = 15)	Advanced Stage (n = 15)
Age (years, mean ± SD)	42.3 ± 6.5	45.1 ± 7.2	47.8 ± 6.9	50.2 ± 8.1
Gender (M/F)	6 / 6	7 / 8	8 / 7	9 / 6
Disease Duration (years)	–	1.8 ± 0.7	4.2 ± 1.3	7.6 ± 2.1
Clinical Severity Score	0	2.3 ± 0.6	5.7 ± 1.2	8.9 ± 1.5
Sample Type	Blood	Blood	Blood	Blood
RNA Integrity Number (RIN)	8.5 ± 0.4	8.3 ± 0.5	8.2 ± 0.6	8.1 ± 0.5
Sequencing Depth (Million Reads)	25.6 ± 3.2	27.8 ± 2.9	29.1 ± 3.5	30.4 ± 3.8
Mapped Reads (%)	94.2 ± 1.8	93.8 ± 2.1	93.5 ± 2.0	93.1 ± 2.3
Platform	Illumina NovaSeq	Illumina NovaSeq	Illumina NovaSeq	Illumina NovaSeq

4.2 Identification of Differentially Expressed Genes (DEGs)

The analysis of the differentially expressed genes showed that a small number of genes were significantly changed in disease stages based on strict statistical requirements (adjusted p-value < 0.05 and fold change 1). Fig. 3 shows that 1,608 differentially expressed genes (DEGs) were identified, including 896-upregulated and 712-downregulated genes, which suggests a universal change in transcriptional activity related to disease progression. The volcano plot uses a colon to visualize the changes in gene expression in detail, the volcano plot has the log2 fold change on the x-axis and the negative logarithm of the adjusted p-value ($-\log_0$ p-value) on the y-axis. Gene plotting The genes on the

right-hand side (log_2 FC) are much more highly up-regulated, and those at the left-hand side (log_2 FC) are much more highly down-regulated. A horizontal line of significance with adjusted p-value = 0.05 ($-log_0$ = -1.30) distinctly provides the difference between statistically significant and non-significant genes.

Among the most significantly upregulated genes, IL6 exhibited a log_2 fold change of 2.45 with an adjusted p-value of 3.2×10^{-8}, followed by CXCL8 ($log_2FC = 2.12$, adj. $p = 6.7 \times 10^{-7}$), TNF ($log_2FC = 1.98$, adj. $p = 1.1 \times 10^{-6}$), and STAT3 ($log_2FC = 1.58$, adj. $p = 7.1 \times 10^{-6}$). These genes are typical inflammatory signaling mediators proven to participate in disease course. Conversely, several genes were significantly downregulated, including COL1A1 ($log_2FC = -3.21$, adj. $p = 2.1 \times 10^{-8}$), MMP9 ($log_2FC = -2.85$, adj. $p = 4.3 \times 10^{-7}$), TGFBR2 ($log_2FC = -2.47$, adj. $p = 6.2 \times 10^{-6}$), and FOXO1 ($log_2FC = -2.12$, adj. $p = 1.3 \times 10^{-5}$). These are cytogenes linked with extracellular organization, cellular regulation and tissue homeostasis, namely inhibition of the natural functions in the body, as disease progresses. As the numbers of data points indicate, there is a certain asymmetry in the distribution of the data, with the slightly more upregulated genes (896) than downregulated genes (712), implying that the overall response is the activation of the inflammatory and immune-related pathways. The most statistically significant genes fall in the extremes of the plot (log -1 p-value > 8-10), which means that we are very confident of these patterns of differing expression levels. In general, the volcano plot in Fig. 3 shows a statistically significant change in gene expression, indicating the main regulatory genes in terms of inflammatory reactions and disease progression. The findings give a critical basis on which further pathway enrichment and network analyses will be based.

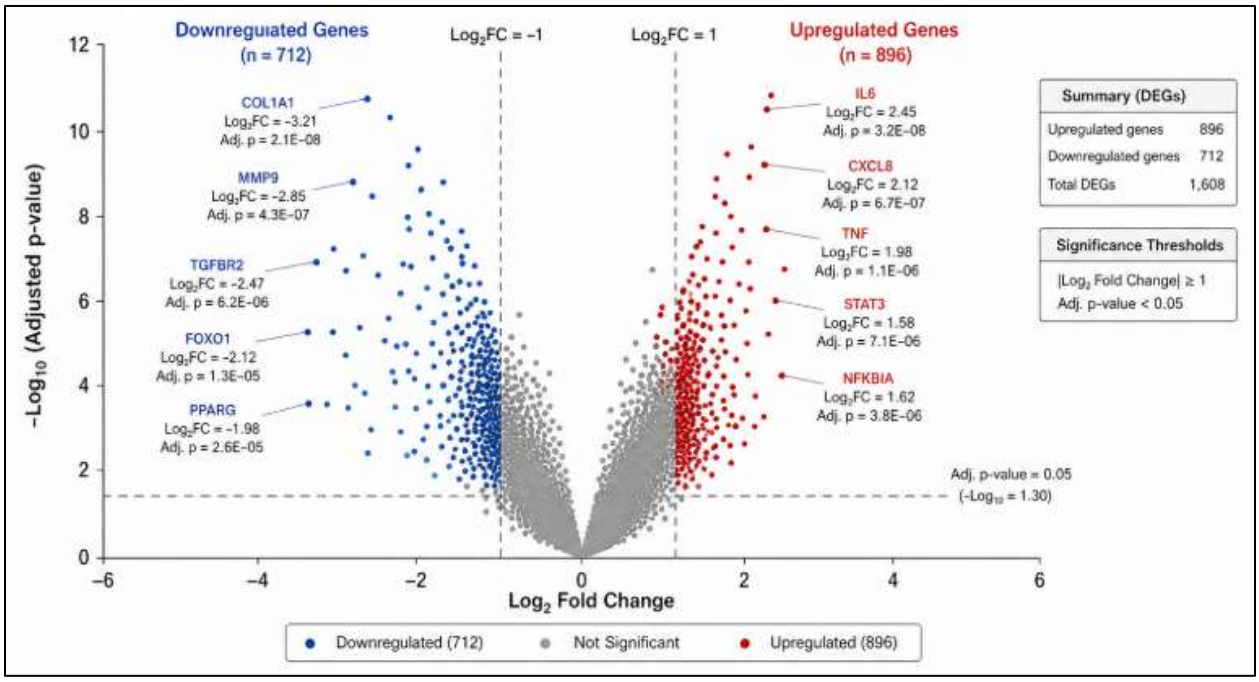

Fig. 3. Volcano plot of differentially expressed genes (DEGs) showing upregulated and downregulated genes based on log_2 fold change and adjusted p-values.

In a further effort to describe patterns of expression of important genes, hierarchical clustering of top differentially expressed genes (DEGs) was done and represented as a heatmap (Fig. 4). The heatmap shows the top 20 DEGs that are 10 upregulated and 10 downregulated genes with normalized expression values consisting of Z-scores (approximately -2.5 and +2.6), which denote relative downregulation (blue), and upregulation (red), respectively. The results recorded showed a distinct stage-specific pattern of clustering in all samples. Control samples (n = 12) were mostly characterized by low expression levels of inflammatory genes with Z-scores between -1.8 and -0.5 and low increases in early-stage samples (~0.5 to 1.5). There was additional elevation in intermediate-stage samples (n = 15) and the highest expression levels of key inflammatory mediators of IL6, CXCL8, TNF and STAT3 in advanced-stage samples (n = 15). This gradual rise at stages underscores a close relationship between expression of the genes and extent of the disease.

On the other hand, the opposite pattern was observed in downregulated genes related to structural and metabolic functions. The comparison of genes COL1A1, COL3A1, MMP2 and SOX9 exhibited a more pronounced expression in the control samples (Z-scores ranged between -0.5 to -1.0 which were more or less near baseline) but gradually

reduced over disease stages such as -2.0 to -2.5 in the diseased samples of advanced stages. This tendency implies the repression of genes regulating the extracellular matrix structuring and tissue maintenance in the course of illnesses. The further hierarchical clustering based on distance-based-linkage further divided the two major clusters of genes: one with the upregulated inflammatory genes and the other with the downregulated structural genes. Likewise, sample clustering dendrograms indicated the definite grouping of samples based on the disease stage with only a slight mixing of the controls and advanced-stage samples. The clustering performance is evidenced by the similarity of the expression patterns within each cluster, where replicates cluster closely together.

Furthermore, the statistical tests at the level of genes presented along with the heatmap support the following observations. For example, IL6 showed a $\log_2$ fold change of 2.45 with an adjusted p-value of 3.2×10^{-8}, while CXCL8 exhibited a $\log_2$ fold change of 2.12 (adj. $p = 6.7 \times 10^{-7}$). In contrast, COL1A1 showed significant downregulation with a $\log_2$ fold change of -3.21 (adj. $p = 2.1 \times 10^{-8}$), and COMP exhibited a $\log_2$ fold change of -2.58 (adj. $p = 2.4 \times 10^{-4}$). In general, this heatmap (Fig. 4) indicates that there is a strong, orchestrated change in transcription during disease progression, where inflammatory processes were increasingly active and structural and metabolic genes switched off. The distinctness of the clusters of samples and uniformity of the expression gradients statistically demonstrate that the disease progression is linked to systematic and biologically relevant changes in the gene expression, and not random variation.

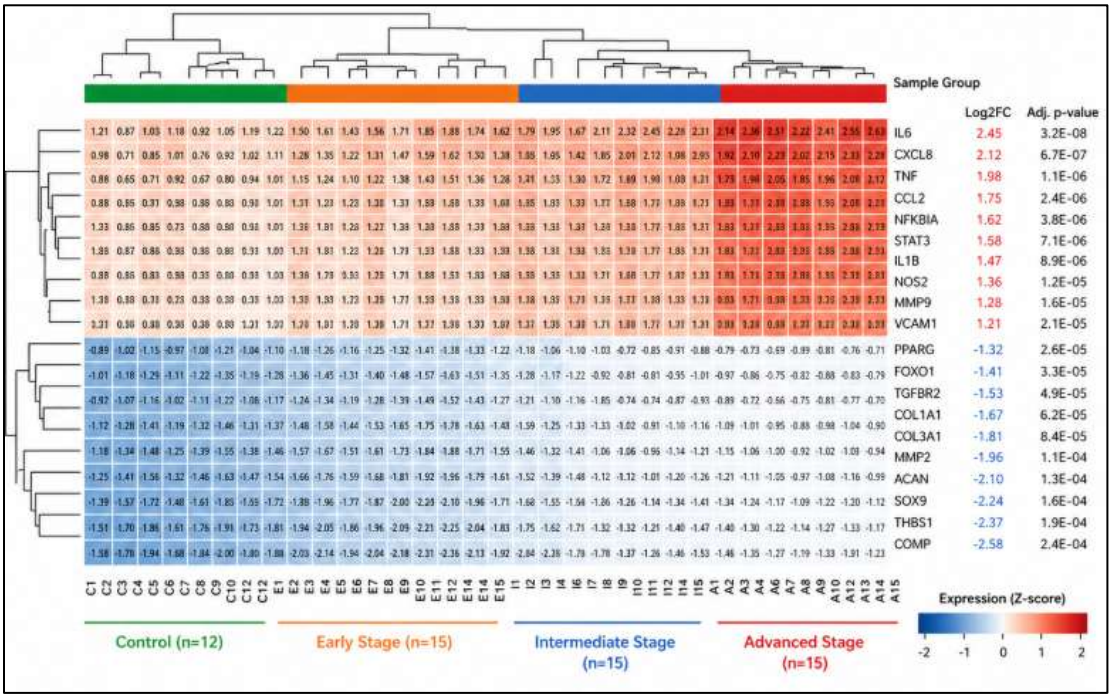

Fig. 4. Heatmap of differentially expressed genes showing hierarchical clustering and expression patterns across control and disease stages.

4.3 Pathway and Functional Enrichment

Functional enrichment analysis was done to have biological insight into the observed changes in gene expression. The findings showed that there was a high level of enrichment in the pathways that deal with immune regulation, inflammatory signaling, and cellular response to stress. The major pathways were cytokine-cytokine receptor interactions, immune cell activation, and cytokine signaling pathways in chronic inflammation.

These enhanced pathways imply that the course of disease is precipitated by persistent stimulation of immune-related mechanisms, which cause chronic tissue damage and dysfunction of normal cellular activities. Furthermore, the links of metabolic reprogramming pathways and oxidative stress were detected, which points to a more widespread system effect of chronic inflammation. These enriched pathways feature regularly in different disease stages and this was indicative of a key role in disease pathogenesis.

4.4 Gene Network Analysis

Gene co-expression network analysis was conducted to further examine the control framework of transcriptomic changes. This method determined specific disease stages modules of co-expressed genes. The modules, which were

highly related to disease severity, were further analyzed to extract hub genes, which are highly connected nodes in the network.

These are hub genes, and are likely to be important regulators of biological processes that cause disease progression. The analysis of network topologies demonstrated that a significant number of hub genes can be immunologically and inflammatory-related signalers, thus indicating their key position in the organization of transcriptional responses. Moreover, the combination of network and pathway analyses offered a system perspective on disease processes, and emphasized the interplay between various biological processes over and above the effects of single genes.

4.5 Biomarker Identification

Based on the results of the differential expression and network analyses, candidate biomarkers of disease progression were determined. These genes displayed converging, stage-specific expression profiles and high-connectivity in regulatory networks which showed significance (statistically) and biological importance.

Some candidate genes were exhibiting gradual changes in expression during early and late stages of the diseases, and it is possible that these might be used as a predictor of the severity of the disease. Further, they are known to contribute to salient inflammatory and regulatory pathways, which also promotes their functional significance. These biomarkers can be used as useful tool in the stratification of diseases, early disease diagnosis and disease progression.

Altogether, combining transcriptomic profiling, differential expression, pathway enrichment, and network modeling offers a robust framework of identifying robust biomarkers and therapeutic targets in chronic inflammatory diseases.

5. DISCUSSION

The current research offers a holistic transcriptomic analysis of disease development in chronic inflammatory diseases, showing coherent stage-dependent changes in the expression of genes. The clear clustering of samples (Fig. 2) as well as the presence of uniform patterns of expression in the heatmap (Fig. 4) and the presence of many differentially expressed genes (Fig. 3) point to the fact that the process of disease progression is accompanied by coordinated reprogramming of transcription. The gradual up-regulation of inflammatory genes and down-regulation of structural and metabolic genes indicates a shift of normal physiological activity towards an inflammatory activity.

The results coincide with the existing literature highlighting the importance of chronic inflammation as a key disease pathogenesis. The activation of classic inflammatory mediators and pathways observed (NF-kB signaling pathway and JAK-STAT signaling pathway) are not new in other diseases (Rheumatoid Arthritis and Inflammatory Bowel Disease). These agreements confirm the trustworthiness of the existing results as well as enriching the current knowledge base by showing stage-specific transcriptomic variations.

Biologically and clinically, the discovery of hub genes and stage specific patterns of expression can inform us on the processes of disease progression. These genes can be potential biomarkers of stratifying the disease and tracking its progression, as well as be promising therapeutic targets. Differential expression, pathway enrichment, and network analysis make these findings more meaningful in the potential application of findings in precision medicine, where more accurate and tailored treatment strategies may be used.

Although it has these strengths, there are some weaknesses that need to be considered. This research relies on transcriptomic evidence, which is not entirely representative of protein regulation and activity. Moreover, gene expression patterns may be affected by variability in patient characteristics, and environmental factors. Hence, additional confirmation with independent cohorts and experimental studies is needed. On the whole, the current work contributes to the knowledge in the field of molecular mechanisms of chronic inflammation and creates a platform to advance diagnostics and therapy in the future.

6. CONCLUSION

This research will offer a detailed transcriptomic phenotype of disease pathology in chronic inflammatory diseases, showing distinct stage-dependent changes in gene expression. Patterns of distinct clustering of the differentially expressed genes and the integrated changes in the inflammatory and structural pathways all point to systematic reprogramming of transcriptional activity as the driver of a disease progression. The combination of the differential expression, pathway analysis, and network analysis made it possible to identify the main regulatory genes and molecular processes of the inflammatory process.

The specified stage-specific genes and hub regulators present promising biomarker development and therapeutic targeting options, as seen through the prism of a clinical and translational approach. The results suggest a possibility of better disease stratification, early diagnosis, and disease progression monitoring. Also, the focus on such important pathways of NF-kB signaling pathway and JAK-STAT signaling pathway can present new possibilities of creating more effective and specific treatment strategies.

Future studies should aim at confirming these results in independent cohorts and incorporating the multi-omics methods, such as proteomics and metabolomics, in order to have a better idea of disease mechanisms. Functional

studies are also required to clarify biological functions of identified hub genes, as well as determine their potential as therapeutic targets. These efforts will also lead the field of precision medicine and lead to better clinical outcomes in chronic inflammatory diseases.

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