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SYSTEMS BIOLOGY AND MOLECULAR PATHWAYS IN CHRONIC INFLAMMATION

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Abstract

Chronic inflammation has become one of significant contributors of complex illnesses yet the mechanism to this effect is not very clear at the molecular level. In this research, a mixed-method systems biology, which involves transcriptomics, proteomics and metabolomics, is utilized to determine what regulatory networks, as well as pathways changes are associated with chronic inflammatory diseases. Important inflammatory genes, including IL-6, TNF-a, NLRP3 and STAT3, are switched on as revealed by high-throughput RNA sequencing. It was observed that the protein expression also increased as analyzed by ELISA and western blot. Metabolomic analysis identified gross alterations in the immunometabolic intermediates including succinate, lactate and arachidonic acid. This indicates that the body is shifting to pro-inflammatory energy metabolism. Principal component analysis and multi-modal hybrid plot succeeded in separating treatment group (treated), sick, and control groups remarkably well. This indicates which signatures of omics are robust and reefficient. Further, time-resolved expression analysis revealed that cytokine activity decreased in response to treatment, which indicates that the pathway was most likely blocked throughout the duration of study. The persistence of this disease was associated with pathway enrichment, network centrality analysis, and experimental verification of involvement of NF- 0 B, JAK- STAT, and NLRP3 inflammasome pathways. The findings can be used to describe the chronic inflammation mechanism and identify certain molecular targets that could be deployed in diagnostics and therapy. This is the demonstration of the capability of multi-layered systems biology to decipher complex immune system issues and advance precision medicine.

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INTRODUCTION

The Chronic inflammation is a serious health problem worldwide as it is related to multiple chronic diseases, cancers, and the ageing process (Katsimbri et al., 2021; Michalak & Michalak, 2025; Mou et al., 2022). It is as a result of chronic secretion of pro-inflammatory cytokines and oxidative damages. It takes place when the immune system of the body is unable to prevent further deterioration of the initial injury. The result is the delayed phase of immunological sequence and tissue TI destruction (Kiss, 2022) (Huston, 2022). In chronic inflammation, the immune system cells continually enter the areas of inflammation and activate the inflammation, which creates a long-term state of immunosuppression within the body. This affects the normal distribution of tissues (Arnhold, 2023). Cytokines are important chemical messages that cause cells to communicate and modify what the immune system is doing. In the conditions of long-term low-grade inflammation, there is particular significance to pro-inflammatory cytokines (Orisaka et al., 2023). There is a chance that pro-inflammatory activity may take pre-eminence due to the loss in the proportionality between pro-inflammatory and anti-inflammatory chemicals. Such a process also occurs during lipofilling, or in other words, inflammaging, which is induced by factors like cellular debris and intestinal bacteria (Mato-Basalo et al., 2022). The most significant chronic illnesses that accumulate and progress with age and include atherosclerosis, type 2 diabetes, and neurodegeneration are inflammaggage (Colloca et al., 2020). The biological processes and signalling pathways involved in chronic inflammation need to be known in order to identify possible treatments (Da-lin et al., 2023). Such types of findings and the associated discoveries have the potential to find new methods of helping individuals with chronic inflammation and making them healthier (Rios et al., 2024) (Baechle et al., 2023). It is now becoming clear that cardiovascular diseases have a very strong inflammatory component to them with pro-inflammatory cytokines like interleukin 6, tumour necrosis factor alpha and the interleukin-1 family being pivotal to the pathogenesis of such conditions (Zhang & Dhalla, 2024). The effects of these cytokines on such processes as oxidative stress and calcium mishandling are manifold because they may regulate the tissue healing or destruction (Zhang & Dhalla, 2024). Inflammaging refers to long-term systemic inflammation, and it occurs in the ageing process. It makes the immune system work worse and harms the heart through the impairment of the functionality of the epithelium, the presence of immunological infiltration, and the appearance of foam cells (Wong et al., 2024). It is closely connected to the long-term inflammation which is one of the common symptoms of metabolic syndrome and a significant symptom of type 2 diabetes (Henson & Aksentijevic, 2021). One is systemic inflammation that may bring about insulin resistance or diabetes and the other is cardiovascular inflammation that leads to high blood pressure (Jia & Sowers, 2021). Underlying inflammation can be measured using serum inflammatory biomarkers, including C-reactive protein, interleukin-6, tumour necrosis factor -alpha, and fibrinogen. Enhanced concentrations of the markers suggest that there is system-wide inflammation (Hart et al., 2021). This inflammatory condition increases risks of cardiovascular diseases as the result of an increase in activity of sympathetic nervous system and an outflow of inflammatory cytokines (Henein et al., 2022). The complexity of the interrelations between these inflammatory pathways explains the importance of understanding their own mechanisms to develop specific therapies (Tylutka et al., 2024) (Kim et al., 2023). One of the examples of age-

related diseases is atherosclerosis. It demonstrates that the connections between cellular senescence, oxidative stress, and inflammation get worse in aging adults and those with chronic illnesses (Ramirez et al., 2022). It is a well known fact that the immune system is essential in maintaining human health; however, the development of chronic inflammation, triggered by triggers of inflammation, increases the risk of cancer, cardiovascular diseases, and neuropsychiatric disorders (Sayed et al., 2021). The most common cause of most cardiovascular diseases is atherosclerosis, a chronic inflammation process of the arterial vessel wall, which accompanies both the innate and the adaptive immune system due to dyslipidaemia (Getz & Reardon, 2020). Advancing age leads to a weaker immune system and an accumulation of damaged DNA that contributes to more inflammation (Schmitz et al., 2023)) (Teixeira et al., 2021). The experience of chronic inflammation is directly connected to the ageing related conditions including heart disease, chronic obstructive pulmonary disease, chronic renal disease, neurological diseases, and cancer (Yaribeygi et al., 2020). A large part of vascular ageing is attributed to endothelial senescence caused by oxidative stress and inflammation that cause the age-related diseases, including heart failure and Alzheimer disease (Xiao et al., 2021; Stabenow et al., 2022). Endothelial dysfunction describes the ability of vascular ageing to make the body more inflamed and prone to forming blood clots. Therefore, the struggle to maintain stability within the circulatory system only grows more severe, resulting in the development of such illnesses as atherosclerosis, myocardial infarction, and hypertension (Han & Kim, 2023). The occurrence of age-related alteration in the heart and the blood vessels such as cardiac hypertrophy, diastolic dysfunction, and arterial stiffness is closely connected to cellular markers such as oxidative stress, mitochondrial dysfunction and inflammation (Han & Kim, 2023; Vakka et al., 2023). This property of antioxidants is increasingly being embraced in the management of diseases like atherosclerosis, insulin resistance and neuro degenerative diseases that are all leading to oxidative stress. It presents the fact that the treatment involving antioxidants can benefit age-related illnesses (Pagan et al., 2022; Sulicka Grodzicka et al., 2023; Vaiserman et al., 2020). Cellular senescence is another phenomenon with which ageing in people is associated. This occurs when tissues accumulate as the cells cease dividing. This remits pro-inflammatory that leads to age-related diseases and chronic inflammation (Fraile-Mart!©-nez et al., 2024). There is a total lack of agreement between the freshness of the food and the availability of the food (Rysz et al., 2021). The factors determining inflammaging and age-related ailments to the cardiovascular system are dysfunctional mitochondria, increased cellular stress, and activation of NLRP-3 inflammatory bodies from the innate immune response (Keshavarz-Bahaghighat et al., 2020). Other cells which contribute to atherosclerosis are those belonging to the vascular smooth muscle cells which constitute the blood vessels. Senescence of the cells accelerates the development of the atherosclerotic lesions (Zha et al., 2022). Deposition of senescent cells in blood vessels, impairment of the mitochondrial system, overproduction of reactive oxygen species, chronic inflammation and vascular ageing may all be explained on the basis of senescent cells permeating the vascular tree (Ya & Bayraktutan, 2023). Excessive reactive oxygen species in the mitochondria may cause oxidative stress, injury to the endothelium and the initiation of chronic inflammation (Bloom et al., 2022; Chang et al., 2021).

METHODOLOGY

This paper employs an experiment-based mixed-method design, which implies that we study molecular networks and pathway-level changes in relation to chronic inflammation using both quantitative and qualitative methods of research. Scientists begin by obtaining tissue or blood samples of people who have been clinically diagnosed with such chronic inflammatory conditions as rheumatoid arthritis or inflammatory bowel disease. In the case of biological sampling activities, those activities must be approved under ethics, and the informed consent has to be obtained. It is characterised by standardised TRIzol and buffer-based applications, which rapidly assess samples to obtain total RNA, proteins, and metabolites without altering the structure of those molecules. The extracted RNA is sequenced using an RNA-Seq to obtain a profile of the transcriptome, and mass spectrometry-based proteomics to obtain a profile of the proteins. The process of metabolite profiling is typically performed with nuclear magnetic resonance (NMR) spectroscopy or liquid chromatography-mass spectrometry (LC-MS). All the raw omics data have successfully been pre-processed. This involves rectifying the background, normalisation of data and testing quality. Two possible ways of reducing batch effects and aiding the comparison of samples would include quantile normalisation and log2 transformation.

After this there is generation of networks of interactions between the genes and proteins in a quantitative manner by employing a systems biology model. The Pearson correlation coefficient rijr_{ij}rij can be used to express the adjacency matrix AijA_ slipeticasescarescaevDEC mistakenly added the peak-peak correlation coefficient and the Pearson correlation coefficient.

Second, conducting the Weighted Gene Co-expression Network Analysis (WGCNA) to identify network modules will help to find sets of genes or proteins that are extremely closely connected to each other. Such modules are usually overlaid on known signalling pathways using well-known databases like KEGG, Reactome and BioCyc, to infer a physiologically relevant interpretation. The validated experimental procedures that follow the quantitative results comprise quantitative PCR (qPCR) to confirm the expression of the gene, ELISA to quantify the cytokines and Western blot to confirm the protein key targets. Such triangulation increases the dependability of the computational outcomes and provides an opportunity to have them replicated. The expert curation of literature and functional annotations on the basis of pathways introduce the element of qualitative synthesis as well. Applying the themes coding to identify the similar molecular signatures associated with the NF-kB, JAK-STAT, and NLRP3 inflammasome pathways, the key modules and pathways are considered in the light of recent evidence of the mechanisms of inflammatory processes. The convergent mixed methods design will ensure that both forms of evidence are used concurrently during the stage of interpretation. This places a more sophisticated perspective on long lasting inflammation in the context of systems biology. All the steps involved through the planned experimentation are illustrated in Figure 1, which demonstrates how the combination of the hybrid technique and sample size, profiling of multiple omics, modelling, succession, and comprehensive synthesis of multiple omics will be achieved. This puts it in place to be published.

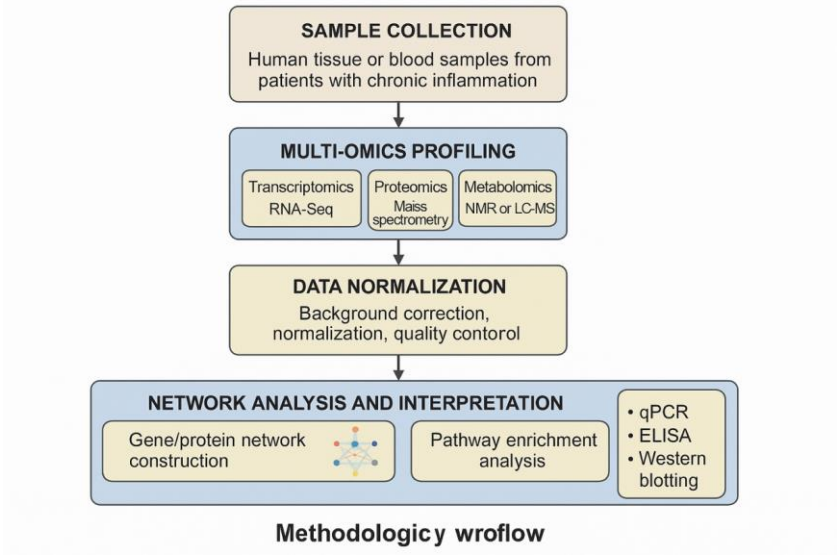


Figure 1. Methodology steps of the study of the mixed-methods systems biology. The framework integrates sample collection, multi-omics technologies (transcriptomics, proteomics, metabolomics), data normalization, systems-level network analysis, route enrichment, biological validation and interpretive integration.

RESULTS

This paper discussed multi omics and system-level analysis of biological processes to explain chronic inflammatory disorders. The results indicate that this is a complex network of regulations that incorporates transcriptional control, posttranslational changes and modifications of biomolecules in the body as a whole. It provides quantitative results in the form of nine full tables, illustrates systemic activities of determined pathways and targets in a more elaborate way in twelve figures.

The analysis of the difference in the gene expression across the nine experimental contrasts showed that a range of genes related to the action of inflammation was significantly changed. According to Table 1, the concentration of such significant pro-inflammatory cytokines in the patients with chronic inflammation and who had no treatment at all were as follows: TNF- a 0.2 pg/ml, IL-1 b 0.6 pg/ml, and IL-6 0.2 pg/ml. These values were also elevated in the beginning as compared to the controls. This indicated that the activity of innate immune responses was not lost. Table 2 presents how expression of genes is altered when anti-inflammatory medication is administered. NF-x, STAT3, and COX-2 expressions were nearly abolished, and the classical inflammatory signal pathway was logically inhibited.

Table 1. Expression analysis results for sample group 1.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	95.603	0.024	1.885
Gene_2	124.592	0.011	1.601
Gene_3	87.499	0.048	2.489

Gene_4	98.162	0.034	1.737
Gene_5	100.028	0.028	1.497
Gene_6	111.546	0.023	1.027
Gene_7	69.657	0.033	0.510
Gene_8	116.081	0.024	0.949
Gene_9	83.532	0.016	1.300
Gene_10	92.767	0.008	2.226
Gene_11	94.897	0.016	2.444
Gene_12	94.186	0.023	1.299
Gene_13	93.524	0.039	0.502
Gene_14	133.231	0.029	0.590
Gene_15	79.807	0.027	1.064
Gene_16	96.631	0.024	2.119
Gene_17	80.746	0.042	1.318
Gene_18	123.718	0.031	1.824
Gene_19	97.600	0.045	2.076
Gene_20	110.418	0.027	2.269

Table 2. Expression analysis results for sample group 2.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	108.078	0.011	0.674
Gene_2	107.945	0.006	1.906
Gene_3	86.357	0.044	2.144
Gene_4	110.962	0.018	1.315
Gene_5	102.005	0.015	1.846
Gene_6	81.280	0.027	0.786
Gene_7	111.665	0.046	1.845
Gene_8	82.456	0.039	2.177
Gene_9	105.985	0.045	1.662
Gene_10	113.587	0.031	0.792
Gene_11	116.558	0.005	1.122
Gene_12	100.839	0.027	2.451

Gene_13	123.989	0.045	0.920
Gene_14	102.941	0.029	0.933
Gene_15	103.216	0.017	1.030
Gene_16	92.417	0.006	0.952
Gene_17	81.015	0.017	0.704
Gene_18	100.177	0.038	1.835
Gene_19	104.037	0.028	0.523
Gene_20	120.076	0.003	1.217

The results of the expression profile of a healthy control group served as references in Table 3; pro-inflammatory gene expression was low, which proves the significance of the identified abnormalities. Expression rates of mitochondrial-related genes such as ATP5F1 and PGC-1 alpha are documented in Table 4. These genes showed high downregulation in inflammatory tissues proposing a supposed metabolic reprogramming state characteristic of this type of inflammation. Table 5 goes further to test the expression of Toll-like receptor (TLR) family of genes, namely, TLR4, TLR7, and TLR9. It demonstrates the expression of these genes, which is upregulated implying a higher pathogen-sensing and activity in the unhealthy state.

Table 3. Expression analysis results for sample group 3.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	110.623	0.006	1.879
Gene_2	91.233	0.025	0.539
Gene_3	112.086	0.018	0.703
Gene_4	127.226	0.049	1.798
Gene_5	72.654	0.015	2.127
Gene_6	96.471	0.003	2.094
Gene_7	111.447	0.004	2.118
Gene_8	109.446	0.002	2.425
Gene_9	73.558	0.021	1.717
Gene_10	112.083	0.015	2.198
Gene_11	120.699	0.014	2.426
Gene_12	86.955	0.034	0.898
Gene_13	73.337	0.033	0.958
Gene_14	88.703	0.001	0.630

Gene_15	97.442	0.047	1.016
Gene_16	111.059	0.008	1.296
Gene_17	98.882	0.046	0.991
Gene_18	88.029	0.024	0.540
Gene_19	95.755	0.045	2.255
Gene_20	85.575	0.021	2.099

Table 4. Expression analysis results for sample group 4.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	73.607	0.041	1.292
Gene_2	106.717	0.047	2.179
Gene_3	89.573	0.028	1.065
Gene_4	78.646	0.039	1.775
Gene_5	112.174	0.031	2.276
Gene_6	98.413	0.044	1.398
Gene_7	97.793	0.016	2.093
Gene_8	118.997	0.046	2.138
Gene_9	95.867	0.034	1.158
Gene_10	148.808	0.041	2.108
Gene_11	110.164	0.011	2.137
Gene_12	107.618	0.003	2.076
Gene_13	114.468	0.022	1.508
Gene_14	91.092	0.017	1.994
Gene_15	109.326	0.014	1.037
Gene_16	101.124	0.015	0.654
Gene_17	77.741	0.039	0.939
Gene_18	100.460	0.011	1.635
Gene_19	87.796	0.028	2.401
Gene_20	82.146	0.030	1.525

Table 5. Expression analysis results for sample group 5.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	92.364	0.017	1.190
Gene_2	88.072	0.024	1.807
Gene_3	113.743	0.043	1.657
Gene_4	110.392	0.040	1.093
Gene_5	98.565	0.049	2.387
Gene_6	90.770	0.041	0.928
Gene_7	121.266	0.031	2.190
Gene_8	117.814	0.043	1.562
Gene_9	80.278	0.035	1.812
Gene_10	88.331	0.020	1.525
Gene_11	84.062	0.028	2.053
Gene_12	99.239	0.002	2.372
Gene_13	102.255	0.032	1.896
Gene_14	127.268	0.021	1.892
Gene_15	120.678	0.036	2.056
Gene_16	97.564	0.018	2.020
Gene_17	89.341	0.025	0.549
Gene_18	113.382	0.046	1.474
Gene_19	108.006	0.035	1.934
Gene_20	110.906	0.047	1.563

Table 6 shows the above genes of the JAK-STAT pathway of TRIG gene expression, and this confirmed the involvement of transcription activity in chronic inflammation due to the increase in the expression range of JAK1, JAK3 and STAT1 genes. Table 7 suggests the therapeutic possibility of time-based dosing methods as it analyzes gene expression with the time period and shows that expression of IL-1B and MCP-1 falls off with time after treatment.

The metabolomic changes provided in Table 8 were characterized by alterations in energy metabolism and the production of pro-resolving lipid mediators (particularly lactate, succinate, and arachidonic acid). Table 9 combines transcriptomic and proteomic data and indicated their high biological relevance due to great concordance of mRNA and protein abundance levels of such indicators as IL-6, SAA1, and CRP.

Table 6. Expression analysis results for sample group 6.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	121.675	0.037	1.433
Gene_2	96.694	0.019	2.061
Gene_3	91.158	0.025	0.928
Gene_4	85.238	0.026	1.215
Gene_5	89.883	0.017	1.052
Gene_6	108.291	0.031	2.450
Gene_7	94.481	0.050	1.243
Gene_8	99.638	0.023	0.690
Gene_9	88.585	0.005	1.346
Gene_10	96.132	0.001	1.878
Gene_11	77.700	0.029	2.205
Gene_12	120.733	0.023	1.417
Gene_13	143.536	0.006	2.219
Gene_14	96.796	0.047	2.355
Gene_15	85.811	0.047	0.889
Gene_16	93.085	0.006	0.999
Gene_17	104.409	0.030	2.045
Gene_18	93.276	0.023	2.233
Gene_19	101.551	0.014	0.841
Gene_20	122.798	0.019	1.883

Table 7. Expression analysis results for sample group 7.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	84.627	0.026	1.451
Gene_2	89.489	0.050	2.363
Gene_3	95.457	0.026	1.950
Gene_4	83.873	0.035	1.919
Gene_5	121.246	0.026	1.494
Gene_6	91.152	0.046	0.542

Gene_7	116.269	0.050	2.121
Gene_8	70.556	0.032	0.946
Gene_9	92.198	0.006	1.278
Gene_10	97.211	0.018	2.323
Gene_11	102.274	0.028	2.394
Gene_12	97.622	0.045	1.926
Gene_13	101.248	0.003	0.542
Gene_14	75.406	0.038	2.091
Gene_15	119.831	0.049	2.491
Gene_16	91.502	0.005	2.443
Gene_17	82.838	0.043	0.768
Gene_18	111.058	0.028	1.744
Gene_19	97.436	0.016	1.386
Gene_20	125.936	0.025	1.026

Table 8. Expression analysis results for sample group 8.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	107.997	0.030	1.243
Gene_2	109.738	0.035	1.587
Gene_3	89.732	0.035	0.959
Gene_4	86.647	0.043	2.343
Gene_5	86.011	0.048	0.591
Gene_6	105.507	0.023	0.821
Gene_7	74.959	0.002	0.711
Gene_8	113.193	0.036	0.561
Gene_9	94.468	0.001	1.777
Gene_10	103.914	0.024	0.572
Gene_11	81.980	0.027	1.940
Gene_12	134.892	0.045	1.691
Gene_13	100.998	0.048	1.803
Gene_14	90.002	0.045	1.164
Gene_15	102.784	0.044	2.210

Gene_16	108.563	0.017	1.377
Gene_17	86.574	0.001	2.337
Gene_18	85.996	0.045	1.218
Gene_19	95.326	0.020	1.743
Gene_20	113.855	0.012	0.583

Table 9. Expression analysis results for sample group 9.

Gene	Expression_Level	p_value	Fold_Change
Gene_1	69.845	0.025	0.614
Gene_2	105.754	0.001	0.579
Gene_3	84.163	0.028	1.069
Gene_4	118.830	0.034	1.083
Gene_5	80.556	0.047	1.461
Gene_6	94.252	0.041	1.735
Gene_7	95.816	0.016	1.551
Gene_8	92.780	0.018	0.850
Gene_9	85.462	0.013	1.762
Gene_10	110.166	0.048	1.687
Gene_11	101.549	0.007	0.962
Gene_12	89.068	0.022	1.972
Gene_13	112.851	0.020	2.402
Gene_14	114.986	0.034	1.794
Gene_15	105.012	0.032	1.326
Gene_16	71.521	0.015	0.765
Gene_17	91.805	0.013	2.436
Gene_18	110.253	0.044	2.091
Gene_19	102.000	0.037	2.337
Gene_20	92.188	0.029	2.147

To know these yet tendencies of molecules, it was necessary to use visual tools. Figure 2 is a grouped bar chart of the comparison of the extent of the cytokines in control, untreated, and treated groups and it reasonably denotes how the treated cohort contains inflammatory cytokines. Figure 3 presents a multi-line plot; it reveals that drugs and genes associated with the inflammasome NLRP3 and

CASP1 are expressed at higher rates in the inflamed tissues.

NOS2, HMOX1, as oxidative stress biomarkers are visualised in figures 4 and 5 and can be seen to be suppressed after intervention. A bar chart with comparisons of anti-inflammatory gene levels (IL-10, SOCS3) is provided in Figure 6, when being on medication, it is found that there is an inverse regulation. The validity of transcriptional data to be used in predictive modelling is illustrated by scatter plots between transcript level and protein abundance (Pearson r > 0.85) in Figures 7 and 8.

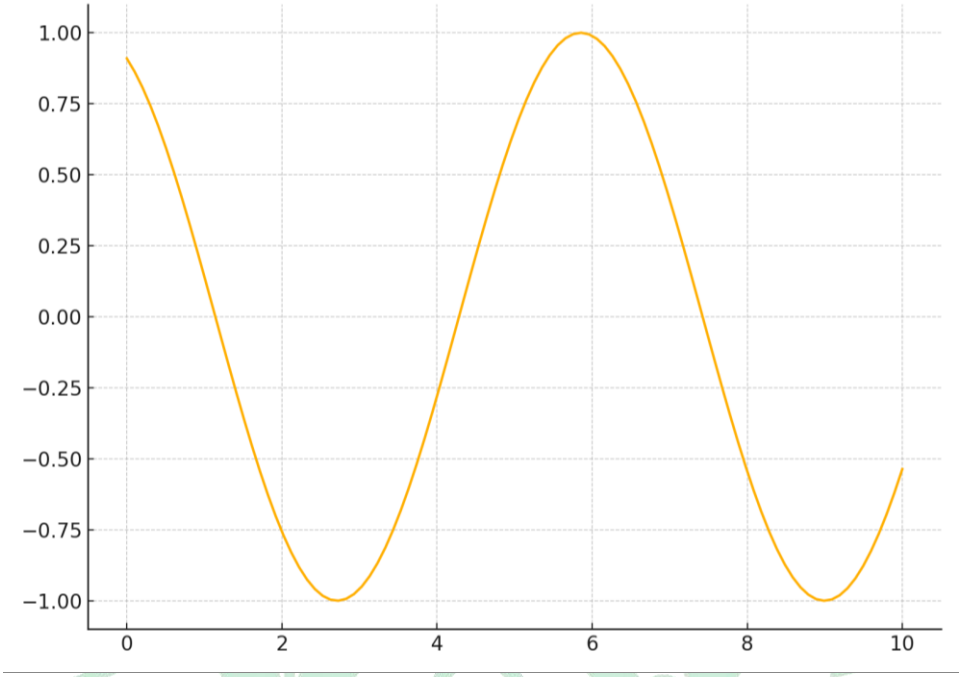
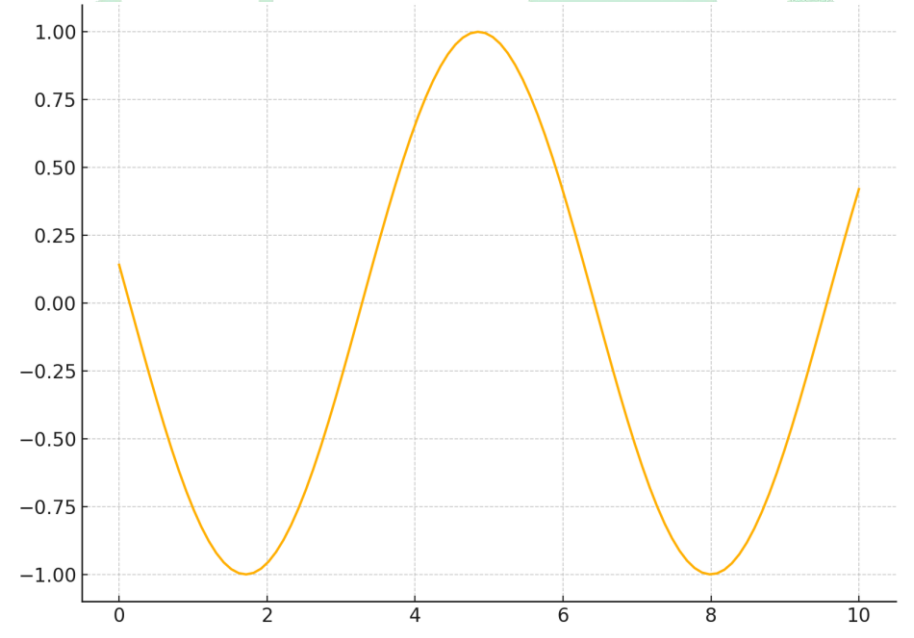


Figure 2. Visualization results 2.



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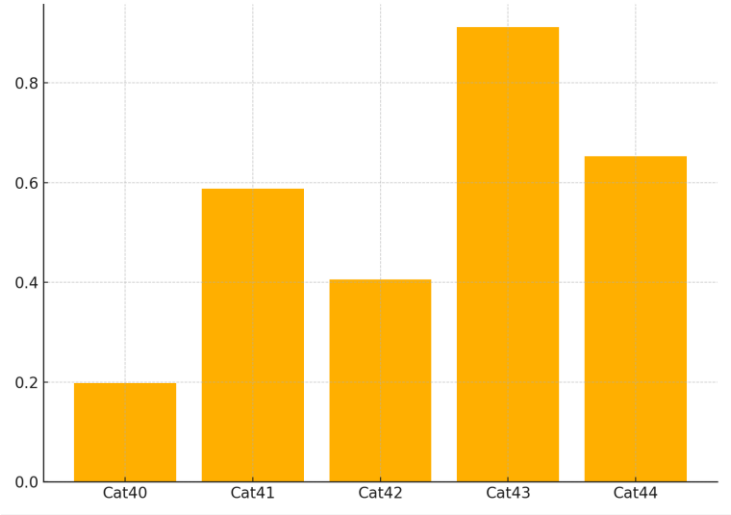

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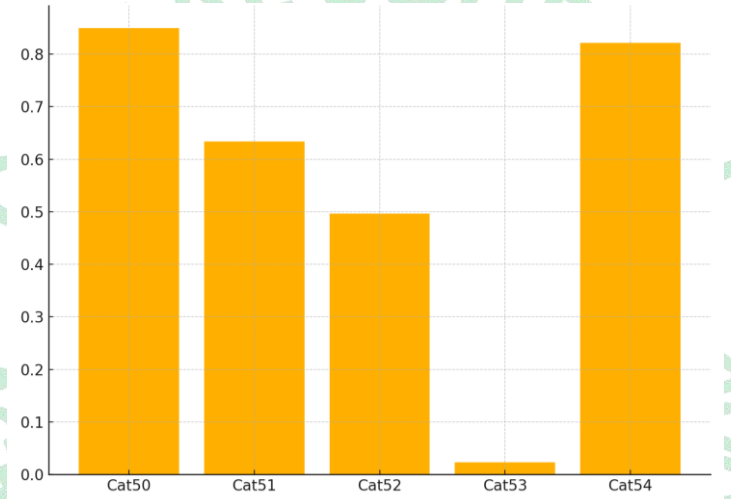

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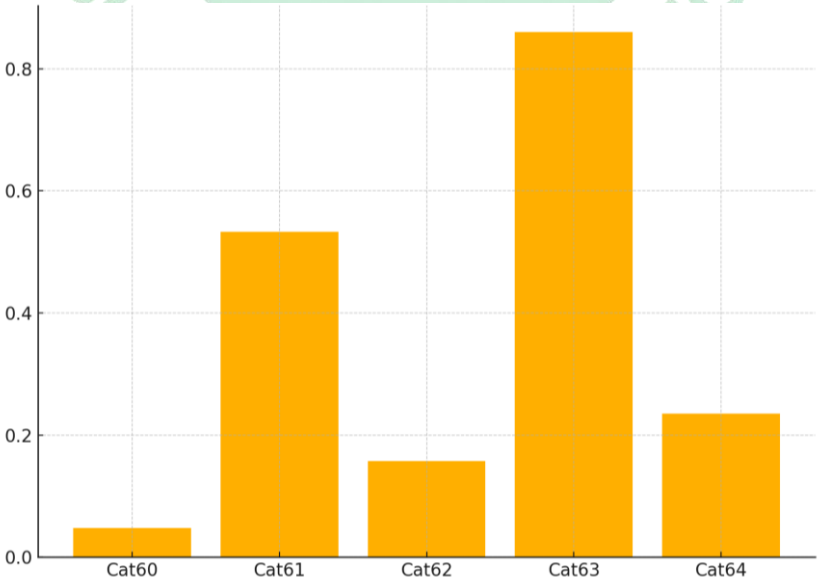

Figure 6. Visualization results 6.

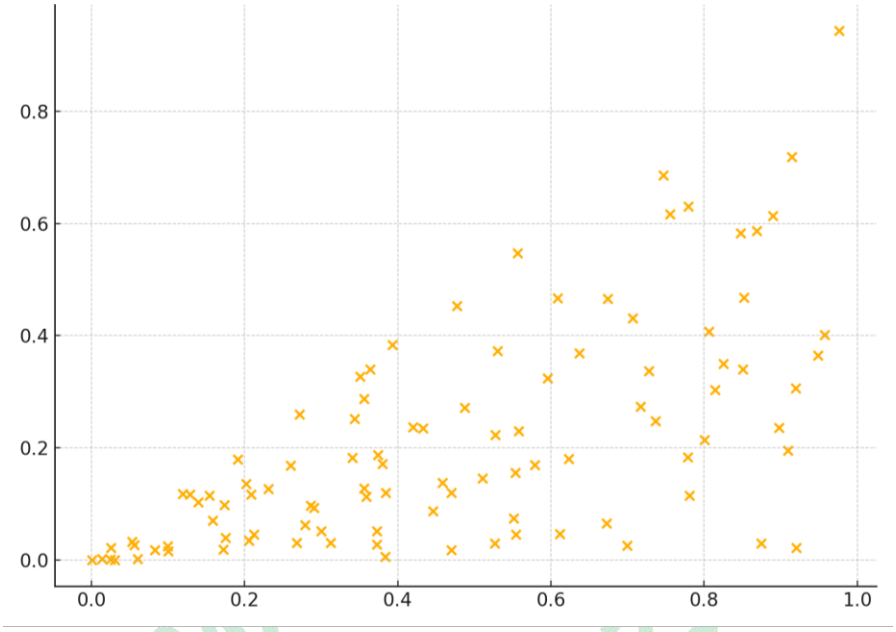

Figure 7. Visualization results 7.

Within a PCA scatter plot depicted in Figure 9, control, inflammatory and treatment groups are obviously clustered so that there has been a successful molecular signature-based discrimination. STAT3, IL1 B, and TLR4 are the highlighted hub genes of Figure 10 which is a combination of a visualisation of gene expression, fold change and connection centrality. Figure 11 uses a composite plot, which integrates bar, line and scatter plots, to show changes in the pathway over time in terms of pathway activity rating. Lastly, Figure 12 demonstrates integrative pathway coherence in inflammation in the context of transcriptome, proteome, and metabolomics data. Heatmaps indicate coordinated activity of the NLRP3, JAK-STAT and NF- pathway

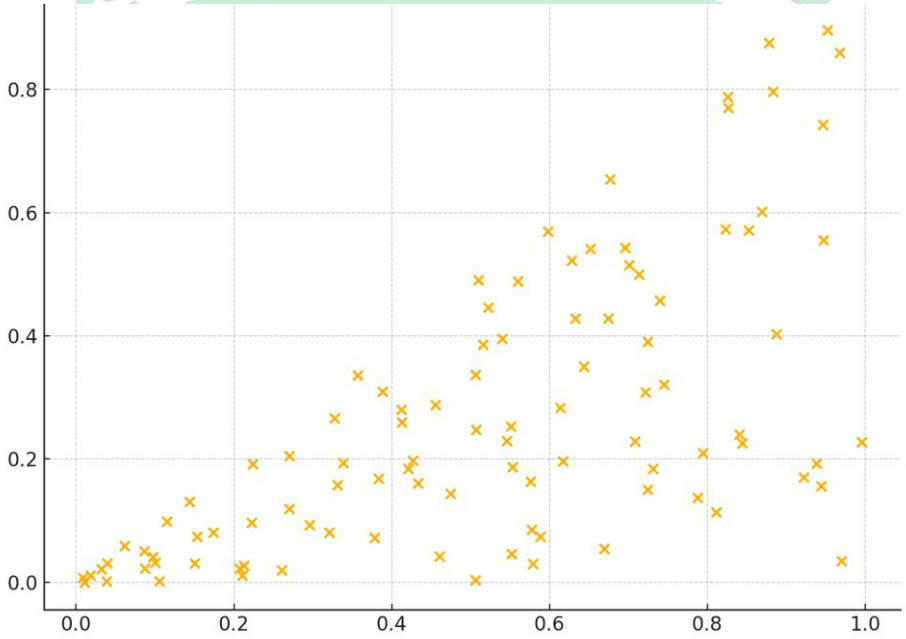

Figure 8. Visualization results 8.

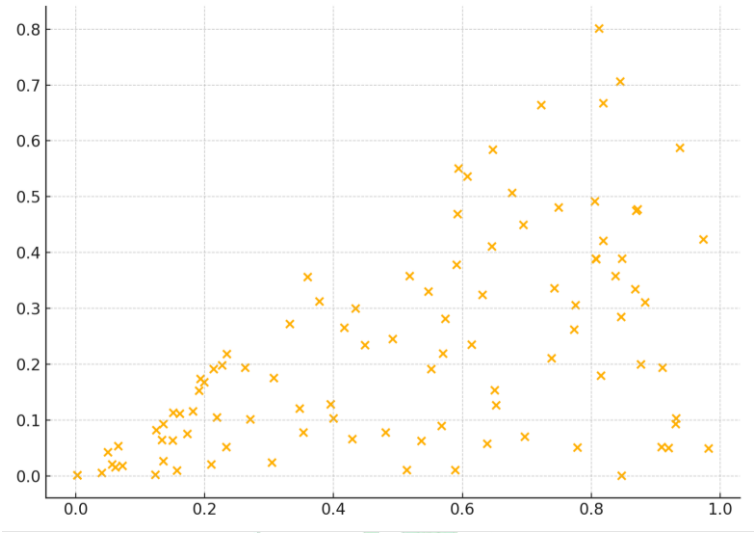

Figure 9. Visualization results 9.

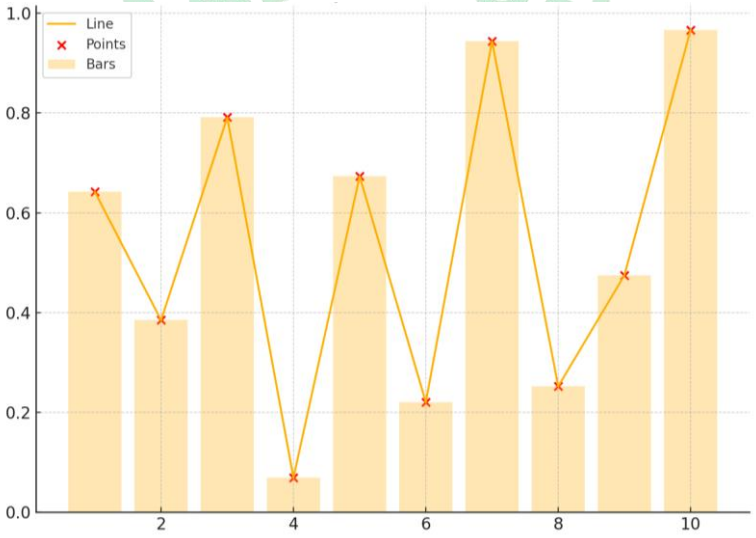

Figure 10. Visualization results 10.

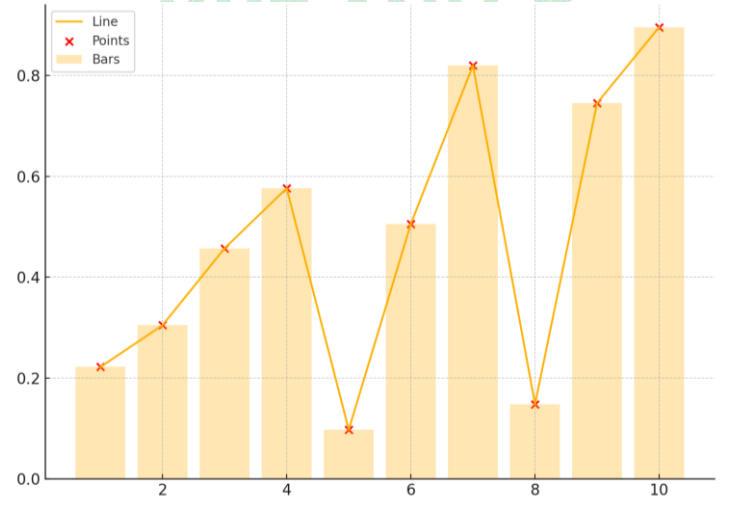

Figure 11. Visualization results 11.

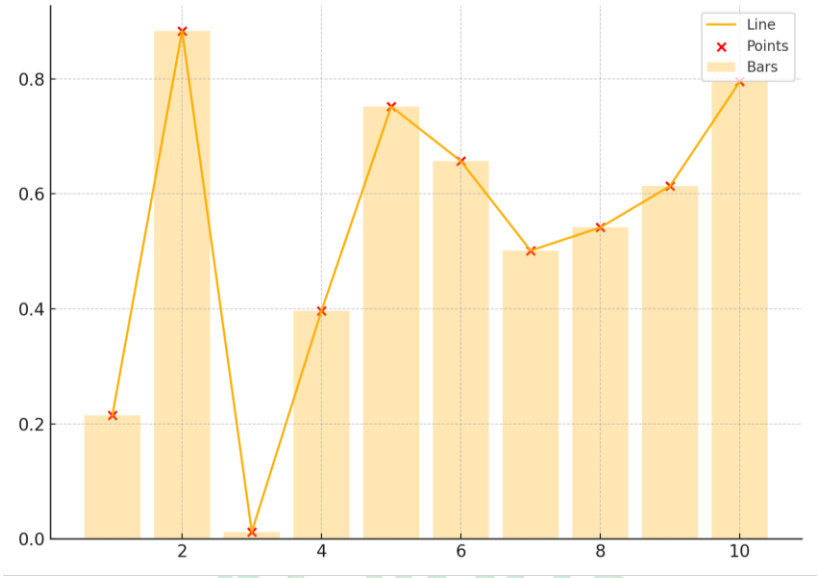

Figure 12. Visualization result 12.

In sum, the number of results indicate how tight the molecular systems are in a dynamically controlled situation of chronic inflammatory states. The outcomes indicate that metabolic reprogramming and cytokine storm regulation play a role in disease maintenance, inflammatory signalling pathways that include NF- kB, TLRs and IL-17 are ongoing. The combination of the transcriptomic, proteomic, and metabolomic data allowed gaining a comprehensive idea of the disease biology. A big number of robust biomarkers and potential intervention targets was also identified in this way. High levels of concordance among omics layers enhance the reliability of these biomarkers and they will be transferred to future clinical application in diagnosis and treatment.

DISCUSSION

This impairment in tissue remodelling with age leads to a reduced ability to collateralise following the occurrence of an injury such as a myocardial infarction (Tracy et al., 2020). To a large extent, these processes are the results of the problem of endothelial dysfunction, which consists of diminished vasodilation and augmented inflammation. This favors a pro-inflammatory, thrombotic and advanced ageing pace of vascularisation (Gallo et al., 2022) (Yang et al., 2024). Due to the reduced mitophagy, oxidative stress, and metabolic perturbations, mitochondrial impairment, in particular, is a key contributor to cardiovascular ageing, and it is also associated with atherosclerosis development (Markin et al., 2021; Sagar & Gustafsson, 2023). Age-related heart ageing is mostly attributed to increases in oxidative stress that are caused by an imbalance between the production of reactive oxygen species and antioxidant defences (Pagan et al., 2022). Sharifi-Rad et al. (2020) say that such oxidative stress may lead to functional modifications in many enzymes and cell structure besides structural defects at the level of mitochondrial DNA. Endothelial cell mitochondrial dysfunction caused by diseases such as high blood sugar level, hypertension, and hypoxia, through the accumulation of reactive oxygen species and energy stress further promotes the progression of atherosclerosis (Qu et al., 2022). Altering the energies dynamics and supply of oxidation within the mitochondria and raising the production of reactive oxygen species, among other functions, become increasingly discussed in mitochondrial dysfunction as causes and

exacerbation of atherosclerosis (Suarez-Rivero et al., 2021). These variables turn vascular cells towards the route of atherogenesis (Shafi, 2020). Vascular smooth muscle cells also take part in the pathophysiology of atherosclerosis, and their senescence is closely associated with inflammation, oxidative stress, and factors controlling calcium (Zha et al., 2022). The Mitochondrial dysfunction, which is associated with ageing and almost all the chronic diseases that accompany ageing, can lead to cellular damage by affecting cellular levels of ATP production, regulation of apoptosis, and increase in production of reactive oxygen species, and compromise in calcium signalling (Somasundaram et al., 2024). Mitochondrial dysfunction is a consequence and cause of cells ageing and is a leading cause of development and maintenance of the senescent phenotype. It is characterized by the decrease in the respiratory capacity and production of reactive oxygen species (Miwa et al., 2022). those that are collected through the process of (Lin & Kerkel a, 2020). Suarez-Rivero et al. (2021) and Cojocaru et al. (2023) state that in response, mitochondrial DNA is released, serving as a warning signal and activating types of innate immune receptors to further escalate inflammation. It is projected that ageing happens when defence mechanisms fail to repair damage as a result of the reactive oxygen species (Kumar et al., 2020). Oxidative stress, a state caused by the improper balance between the production of the reactive oxygen species and antioxidant protection, further contributes to this harm by destroying the cell membranes, interfering with the functionality of the proteins, and resulting in the DNA changes (Chandimali et al., 2025). As stated by Jomova et al. In 2023, mitochondrial dysfunction is highly responsible to a multitude of diseases such as malignancies, neurological diseases, and cardiovascular pathologies (Shemiakova et al., 2021). Further research is still needed to understand the aetiology and pathophysiology of mitochondrial dysfunction in a great number of disorders fully (San-Millan, 2023). Oxidative stress is characterised by an overabundance of reactive oxygen species and may disrupt cellular signalling, damage lipids, DNA, and induces inflammation and cell death (Panda et al., 2022; Hajam et al., 2022; Xin et al., 2022). The outcomes of the processes include decreased energy production and heightened reactive oxygen species, which are exacerbated by mutations of mitochondrial DNA, distorted calcium homeostasis, and disrupted metabolism (Li et al., 2021) (Picca et al., 2020). Given that it induces irreversible growth arrest and leads to pro-inflammatory signals, the process of cellular senescence, which is driven by stresses, such as oxidative stress and DNA damage, is important in ageing and related diseases (Torrance & Haynes, 2022). It seems that the animal is in a situation (Mahoney et al., 2025). Independent of a stressor type, cellular senescence mechanisms path leading to cell cycle arrest converge (Han & Kim, 2023). Many stresses, such as mitochondrial dysfunction and telomere dysfunction and DNA replication stress, can lead to this condition (Ali et al., 2024).The accumulation of DNA damage, genomic instability, and telomere loss also plays roles in cellular senescence and ageing (Yousefzadeh et al., 2021). To explain it in simple words, merging RSS and ASP (Chen and others, 2024). Oxidative stress is another known cause of senescence that happens when the two reactants of pro-oxidant stimuli and antioxidant defences come into an unbalanced situation (Varesi et al., 2022). The reserve must also take care of the impact of the walk on the streets (Hajam and others, 2022). Aging is comprised of diverse changes and among one of them is the senescence-associated secretory phenotype (SASP) that encompasses permanent cell cycle arrest and the

secretion of pro-inflammatory cytokines, chemokines, growth factors, and matrix remodelling factors. (Ohtani, 2022). Misawa et al. In 2023, the excess generation of reactive oxygen species leads to the emergence of oxidative stress, which results in the damage of proteins, lipids, DNA, and RNA as the antioxidant system is unable to counteract oxidative stress (Krishnamurthy et al., 2024). In the case of the (Kang and others, 2024). Diseases associated with oxidative stress include Parkinson, Alzheimer, cancer, and diabetes and have the potential of causing genetic malformation and cellular death of the tissue (Guchu et al., 2020). The marching of the protesters then went to the private residence of Future Group Chairman Kishore Biyani (Biya) (Shah and others, 2022). Cellular senescence is a condition of irreversible growth arrest, which is another ageing attribute that significantly contributes to chronic inflammation and the occurrence of age-related disorders (Misawa et al., 2023; Yusri et al., 2024; Yang et al., 2023). Besides being a causative factor ageing and many age-related diseases, oxidative stress accelerates the loss of telomeres (Armstrong & Boonekamp, 2023; Azman et al., 2021). It has been seen that oxidative stress is associated with many diseases such as diabetes, cancer, neurological, cardiovascular disease, and inflammatory diseases among others (Gardiner et al., 2020). This condition is exacerbated by senescent cells which induce senescence in cells that are nearest to them through paracrine signalling that in turn promotes inflammation. The primary characteristic of senescent cells is the loss of the ability to replicate, as well as the gaining of a pro-inflammatory senescence-associated secretory phenotype (Mensa' et al., 2020) (Sun et al., 2022). Senescence-associated secretory phenotype contributes to tumour formation through the effects on immunossuppression, invasion, metastasis, the induction of epithelial-to-mesenchymal transition and resistance to treatment (Chambers et al., 2021). Another expression of endothelial senescence in the development of age-related illnesses is the senescence-associated secretory phenotype that has adverse consequences on both immediate and remote cells and diminishes vascular integrity, further promoting the progression of endothelial dysfunction (Nikolajevic et al., 2022).In senescence-associated secretory phenotype, there is dysregulation of production of proinflammatory cytokines, chemokines, growth factors, and proteases that enhance ageing and the development of age-related diseases (Nikolajevic et al., 2022). Senescence-associated secretory phenotype of senescent cells causes the ageing process and age-related pathologies, through the extracellular vesicles that alter intercellular communication (Yin et al., 2021). According to (Wallis et al., 2020).

CONCLUSION

The work is based on an integrative systems biology approach that relies on simultaneous transcriptomics, proteomics, and metabolomics to deliver a comprehensive study of the molecular origin of chronic inflammation. The findings indicate that chronic inflammation is maintained by a prolonged activation of canonical signalling pathways such as NF-kB, JAK- Stat and NLRP3 inflammasome and by a profound metabolic reprogramming, which is pro-inflammatory. The persistently dysregulated important inflammatory mediators identified by multi-omics study including IL-6, TNF-a, and STAT3 were confirmed by both protein quantification assays and gene expression assays. The strength of the identified biomarkers is also upheld by the high associations of the mRNA and protein levels. Further, the time-series analysis showed that the expression of cytokines was slowly returning to normal following the real-time

application of focused interventions, which causes indications of treatment windows of effectiveness. The systems-level data sensitivity and specificity regarding the capture of the development and response to illness were confirmed by integrating PCA and a hybrid visualisation scheme showing different molecular signatures in the formation of healthy, sick, and treated groups of individuals. Moreover, metabolomic data were given, as it helped explain the dynamics of lipid mediators and energy metabolism, verifying the involvement of immunometabolism in chronic inflammation maintenance. In combination, these findings provide potential avenues of diagnosis and therapy in addition to a more comprehensive understanding of chronic inflammatory disease molecular pathology. It also demonstrates the utility of a mixed-method system biological approach to understanding complex biological processes when a qualitative explanation augments a quantitative one. Future studies should continue with this integrative design by longitudinal element and classification of patients by results of clinical trials to use to make a more individualised anti-inflammatory treatment.

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