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Uncovering dendritic cell specific biomarkers for diagnosis and prognosis of cardiomyopathy using single cell RNA sequencing and comprehensive bioinformatics analysis

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Cardiomyopathy is a type of cardiovascular disorder that is a primary cause of death globally, killing millions of people each year. Cardiomyopathy detection and early diagnosis are crucial in reducing negative health effects. Thus, this study aims to use single cell RNA sequencing, and bioinformatics analysis to uncover dendritic cell-specific biomarkers, gene ontology, pathways, regulatory interaction networks, and protein-chemical compounds related to the molecular mechanism of cardiomyopathy progression. Two RNAseq datasets GSE65446 and GSE155495 also were evaluated to identify significant biomarkers in cardiomyopathy, and 123 mutual DEGs appeared between scRNAseq and RNAseq datasets. In addition, the DAVID online platform and FunRich software were utilized to detect cell communication in innate immune responses, type 1 IFN, antigen processing and presentation, allograft rejection and viral infection significant gene ontology and metabolic pathways in cardiomyopathy. The protein-protein interaction (PPI) network revealed five key hub proteins (ITGAX, IRF7, MX1, HLA-B, and IRF1). Following that, several transcription factors (GATA2, FOXC1, SREBF1, STAT3, and NFKB1) as well as microRNA (hsa-mir-26a-5p, hsa-mir-129-2-3p, etc.) were predicted. Prospective chemical substances such as tretinoin, valproic acid, and arsenic trioxide have been predicted to be linked to cardiomyopathy treatment. The acceptable value of receiver operating characteristic (ROC) curve analysis revealed that biomarkers play critical roles in cardiomyopathy. This study identifies molecular indicators at the RNA and protein levels that may be useful in improving understanding of molecular causes, early diagnosis, and devising favorable cardiomyopathy treatment. More research will be needed to validate our predicted findings as future clinical biomarkers.

Keywords Single-cell RNA-sequencing, Cardiomyopathy, Networking analysis, Biomarkers, And Bioinformatics

Cardiomyopathies, which are a commonly occurring set of genetic heart diseases, are the primary cause of heart failure and unexpected cardiac demise. Cardiomyopathy has five different subgroups including dilated

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cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), left ventricular non-compaction (LVNC), restricted cardiomyopathy (RCM), and arrhythmogenic cardiomyopathy (ACM) that exhibit phenotypic variations[1]. Although HCM and DCM are among the most prevalent forms of cardiomyopathies, they differ in their underlying causes and clinical features. These cardiomyopathies can develop in a variety of medical conditions, some of which are considered unusual or rare. These may also be linked to arrhythmias, which are irregular cardiac rhythms that can be life-threatening in some circumstances[2]. Ventricular hypertrophy and systolic dysfunction can occur in the absence of apparent aberrant loading circumstances or substantial coronary artery disease[3]. It is widely believed to be a primary contributing factor to the global incidence of heart failure. The prevalence of DCM in Europe and North America is shown to be 36 cases per 100,000 individuals, with an annual incidence ranging from 5 to 7.9 occurrences per 100,000 individuals. According to available data, Japan has a higher prevalence of DCM compared to Europe, with around 14 reported cases per 100,000 individuals[4]. Cardiomyopathy, which causes severe morbidity and mortality, is thought to develop in 5.7 million persons (20–30%) of those with chronic Chagas infection in Latin America[5]. While up to 19% of heart disease cases have been observed to have restrictive cardiomyopathy in tropical regions of Africa[6]. This observation implies that DCM may be more prevalent in regions such as Africa and Latin America, as opposed to Eastern Asia due to endemic infectious diseases and differing healthcare systems.

Paediatric cardiomyopathy is linked to several risk factors including genetics (have a major impact on hypertrophic and dilated cardiomyopathies)[7] and environmental factors (such as viral infections and toxins)[8]. As a result, the reaction to myocardial injury in juvenile cardiomyopathies is gradually being achieved through primary, translational, and clinical investigations[9]. Numerous genetic predispositions (HLA-DR4), metabolic, energetic, and contractile abnormalities (PKC-α) in cardiomyocytes, cardiac C-protein, SERCA2a, phospholamban, and aberrant modulation of cellular immune responses as demonstrated by activated blood T-lymphocytes and macrophages[10,11]. Before cardiac failure, myocardial fibrosis is present when there are mutations in the PKP2 (plakophilin-2), DSC2 (desmocollin-2), DSG2 (desmoglein-2) and DSP genes[12–14]. Mechanosensing pathways including the classic Wnt and Hippo pathways are implicated in the pathogenesis of ACM. Besides, the behavior of cardiac cells such as their proliferation, apoptosis, and cellular adhesion is largely regulated by these signalling pathways [15,16]. There is a substantial overlap in the genes causing the different subtypes of cardiomyopathy. Furthermore, variations in over 100 genes have been connected to cardiomyopathy[17,18]. This knowledge is essential for the advancement of patient treatment strategies and the optimization of clinical outcomes[19].

In addition, dendritic cells (DC) are a type of immune cell involved in initiating and regulating immune responses which may be involved in the autoimmune process leading to cardiac inflammation and damage[20]. Heart resident interstitial CD11c + cells appear to be essential for the propagation and maintenance of heart failure with autoimmune origins and animal studies imply that DCs play a significant role in the production of autoimmune myocarditis[21]. Researchers demonstrated that chronic DC-induced myocardial inflammation has been linked to ventricular dysfunction and DCM-like hemodynamics[22]. Interestingly, there is currently a lack of thorough research that has investigated whether unfavorable remodeling happens in children or has pinpointed the specific age at which adverse remodeling begins in adults. While certain individuals may develop clinical cardiomyopathy throughout their youth, others may not manifest symptoms until they reach adulthood[23]. However, the specific molecular contribution of each dendritic cell type to the onset of the disease is yet uncertain. The abundance of data obtained from genomic and proteomic investigations has not been effectively applied in clinical practice. Besides, a better understanding is still needed of the biological knowledge and connections related to cardiomyopathy, cell-specific biomarkers that can offer reliable diagnostic and prognostic value, as well as high specificity and sensitivity, with a meaningful impact on clinical practice[24].

Single cell RNA sequencing (scRNA-seq) technology, a recent innovation has been utilized to detect and assess gene expression patterns in individual cells. This can greatly assist researchers in identifying novel biomarkers[25]. ScRNA-seq, unlike bulk RNA sequencing involves analyzing individual cells from many tissues to discover new biomarkers and categorize cell types[26,27]. This current study aims to identify dendritic cell specific potential biomarkers that reflect the associated factors' alignments related to the development of cardiomyopathy in the heart by scRNA-seq and RNAseq data analysis. In addition, we have identified potential key hub proteins from protein-protein interaction (PPI) network that are expressed at different levels, as well as the pathways that are associated with cardiomyopathy. By identifying transcriptional and post-transcriptional regulators, we sought to explore molecular biomarkers for the early detection of cardiomyopathy and its progression. We also performed a receiver operating characteristic (ROC) analysis to validate the results in clinically. Concurrently, we sought to identify prominent gene list components that may interact with one another to provide useful drugs or targets for future research.

Results

Datasets processing, normalization, clustering and annotation

During the data read process, we employed first quality control to filter out low-quality cells with fewer than 200 expressed features (genes) and lowly expressed features in less than three cells. We eliminated instances with zero counts and took 10,000 read counts from the entire gene expression matrix to show gene expression patterns before and during normalization. Following cluster analysis, a UMAP graphic (Fig. S1 and Table S1) shows the 28 scRNA-seq data clusters that have been identified using reference-based annotation. Next, five distinct sets of cell markers were examined, including T cells, B cells, monocytes, dendritic cells, and natural killer cells. Several subgroups have been produced by those five cell markers. We have only chosen the dendritic cell clusters (21, 22 and 24 numbers) for the scRNAseq dataset. Because dendritic cells play a significant role in the production of autoimmune myocarditis.

Identification of differentially expressed genes (DEGs) by statistical analysis

We have found 1838, 1783 and 2370 DEGs from 21, 22 and 24 clusters of dendritic cells, respectively, by setting a cut-off range ($P < 0.05$, and $|\log2FC| > 1$). By removing duplicate DEGs and merging all the identified three clusters of dendritic cells, we have obtained 3649 distinct DEGs in cardiomyopathy patients of scRNAseq dataset.

On the other hand, we have found 5807 and 2584 differentially expressed genes (DEGs) from GSE65446 and GSE155495 datasets of RNAseq, respectively by comparing control and cardiomyopathy samples from GREIN database by setting a cut-off range ($P < 0.05$, and $|\log2FC| > 1$). After that we discovered 871 reciprocal DEGs between the two RNA-seq datasets. Based on the Venn diagram, there are 123 shared DEGs identified among the dendritic cells (scRNA-seq), GSE65446 and GSE155495 datasets for further analysis, as shown in Fig. 1.

Evaluation of functional enrichment and pathway analysis of shared DEGs

The functional enrichment studies about the differentially expressed genes (DEGs) were conducted using the FunRich tool to facilitate further investigation. Figure 2 illustrates the top GO enrichment functions of 123 DEGs. Genes related to signal transduction accounted for 73.5% of the enrichment in biological processes (BP), followed by genes related to cell communication (71.4%) and immune response (20.4%) as shown in Fig. 2 and Table S2. Genes that were abundant in the plasma membrane (60.8% of the cellular component); exosomes (43.2%); and integral to the plasma membrane (20.3%) were enriched in the cellular component (CC) as shown in Fig. 2 and Table S2. The analysis of molecular function (MF) revealed that 31% of the genes were enriched in receptor activity, 20.7% in both MHC class I and class II receptor activity, over 17% in calcium ion binding, and 13.8% in GTPase activator activity as shown in Fig. 2 and supplementary Table S2.

From three databases including KEGG, Reactome, and WiKi, the most affected pathways of frequent DEGs of cardiomyopathy were compiled and presented in Fig. 3 and supplementary Table S3. The primary findings of the study were obtained by selecting p-values < 0.05. The KEGG analysis reveals that the top enriched pathways include infections with the Epstein-Barr virus, Human papillomavirus, Herpes simplex virus 1, Phagosome, Kaposi sarcoma-associated herpesvirus, Human immunodeficiency virus 1, Human T-cell leukemia virus 1, Human cytomegalovirus infection, and Human immunodeficiency virus 1, processing and presentation of antigens, molecules that bind cells as shown in Fig. 3. Immune system, cytokine signaling in the immune system, neutrophil degranulation, and interferon alpha/beta signaling the most enriched pathways for Reactome pathways include Antigen Processing-Cross presentation, Endosomal/Vacuolar route, ER-Phagosome pathway, Interferon gamma signaling, and Innate Immune System as shown in Fig. 3. In the WiKipathways, TYROBP causal network in microglia, Ebola virus infection in host, Allograft rejection, Proteasome degradation, Burn wound healing, Regulatory circuits of the STAT3 signaling pathway, non-genomic actions of 1, 25 dihydroxyvitamin D3, Measles virus infection, Type II interferon signaling are some of the top enriched pathways shown in Fig. 3.

Hub protein selection from the PPI network of shared DEGs

A comprehensive set of 123 differentially expressed genes (DEGs) were employed in the PPI network analysis. A confidence score of 400 was ascribed to the STRING Interactome. This network consisted of 121 nodes and 220 edges, resulting in an average node degree of 3.64. The PPI enrichment analysis revealed a very significant P-value of less than 0.05, as shown in Fig. 4, based on the online tool STRING.

Using the 10 different network scoring methods including Betweenness, Stress, MNC, MCC, EPC, Radiality, DMNC, Degree, Closeness, and BottleNeck in the cytoHubba plugin of Cytoscape, the top 15 genes were chosen. After that, we identified five common key hub proteins such as ITGAX, IRF1, IRF7, MX1, and HLA-B among

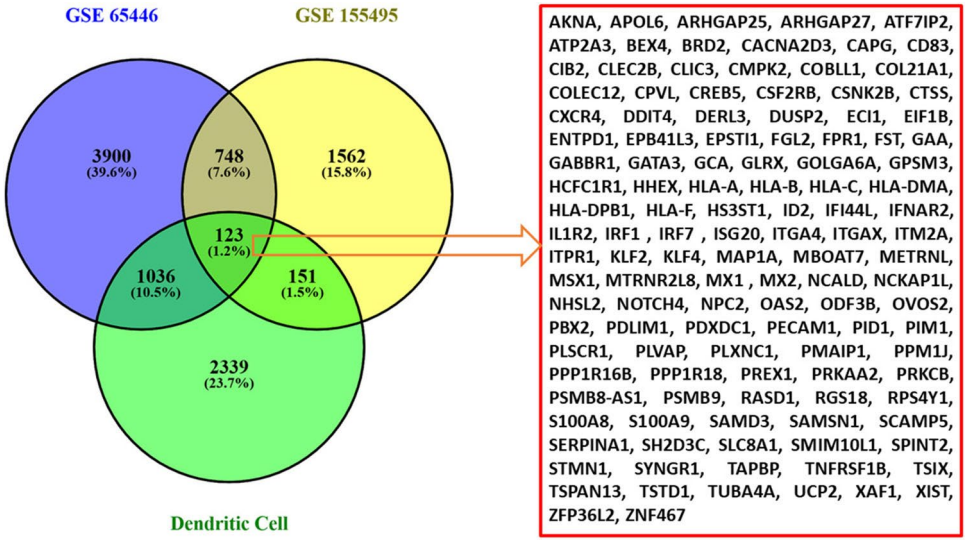

Figure 1. The Common DEGs representation through a Venn diagram. List of significant 123 shared DEGs between dendritic cells (merge DEGs of three denoted clusters) of scRNA-seq dataset and cardiomyopathy of two RNAseq datasets.

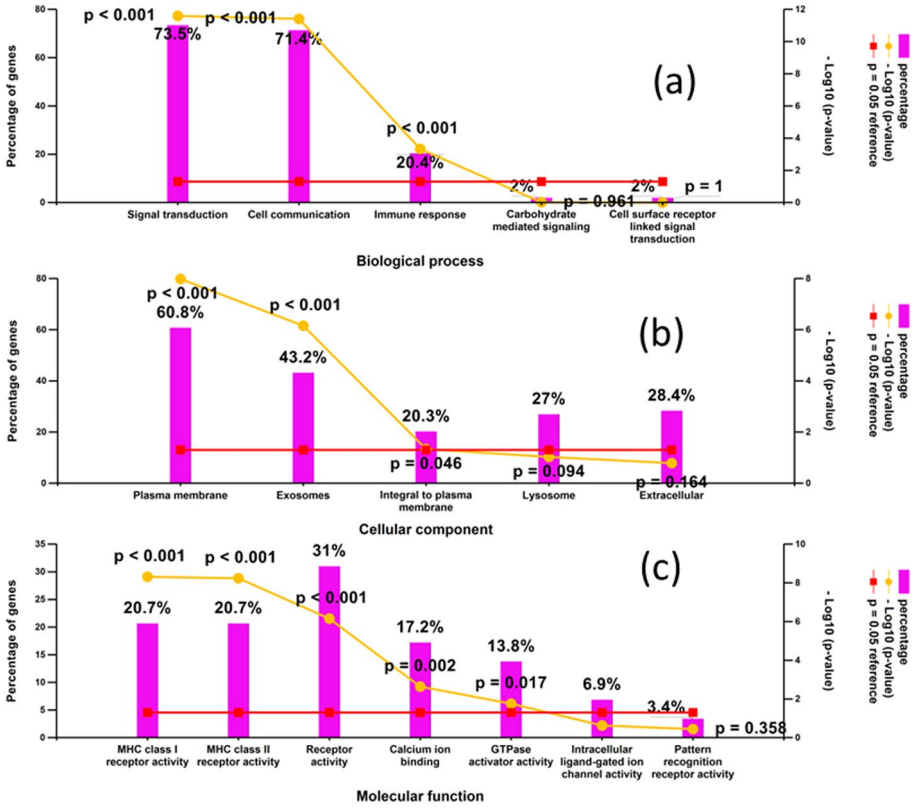

Figure 2. The top enriched GO terms were closely related to cardiomyopathy enriched by DEGs between the low and high-risk groups in (**a**) Biological processes, (**b**) Cellular components, and (**c**) Molecular functions.

the top fifteen genes of each of the above approaches. We identified the hub proteins in the Upset plot that span seven or more methods illustrated in Fig. 5.

Correlation between key hub proteins and significant pathways

The core goals of ID were directly associated with the top eight GO words, which included the most important terms from the ontologies of BP, CC, and MF. These GO terms encompassed various biological processes such as the interferon-gamma-mediated signaling pathway, defense response to virus, and innate immune response. Additionally, the core targets were found to be localized in cellular components such as the cytoplasm, secretory granule membrane, and membrane. Furthermore, the core targets were involved in molecular functions such as protein binding and peptide antigen binding, as indicated in Fig. 6. On the other hand, as depicted in Fig. 7, the core targets of infectious diseases are linked to the top 8 molecular pathways, namely human papillomavirus infection, cytokine signaling in immune system, TYROBP causal network in microglia, herpes simplex virus 1 infection, Type II interferon signaling, immune system, measles virus infection, and interferon signaling.

Analyzing the relationship between TFs and miRNAs

NetworkAnalyst's JASPAR database was used to build the key hub proteins and TFs interaction networks. As transcriptional regulatory factors of key hub proteins, five TFs (GATA2, FOXC1, SREBF1, STAT3, and NFKB1) with degrees > = 3 were chosen as shown in Fig. 8.

To identify the microRNAs (miRNAs) that interacted with the key hub proteins, we included the official symbol into the gene list of human heart tissue using the TarBase database in NetworkAnalyst, depicted in Fig. 8. Nine essential miRNAs (hsa-mir-26a-5p, hsa-mir-129-2-3p, hsa-mir-21-3p, hsa-mir-27a-5p, hsa-mir-212-3p, hsa-mir-221-3p, hsa-mir-133a-3p, hsa-mir-200b-3p, and hsa-mir-34a-5p) were chosen as post-transcriptional regulatory factors of hub genes based on degree score > = 3. We discovered from the literature study that there is a strong correlation between these miRNAs and cardiomyopathy's medication resistance.

Protein–chemical compounds (PCI) interactions of key hub proteins

The protein-chemical interaction networks of cardiomyopathy were found in this experiment. Over-all about thirteen chemical substances were identified as possibly associated. The substances encompassed in the aforementioned list are tretinoin, arsenic trioxide, benzo (a)pyrene, decitabine, nickel, tetrachlorodibenzodioxin, valproic acid, antirheumatic agents, monomethylarsonous acid, silicon dioxide, C646 compound, ICG 001, and nickel sulfate. Figure 8. demonstrates the PCI, where the chemicals are represented by hexagon shapes.

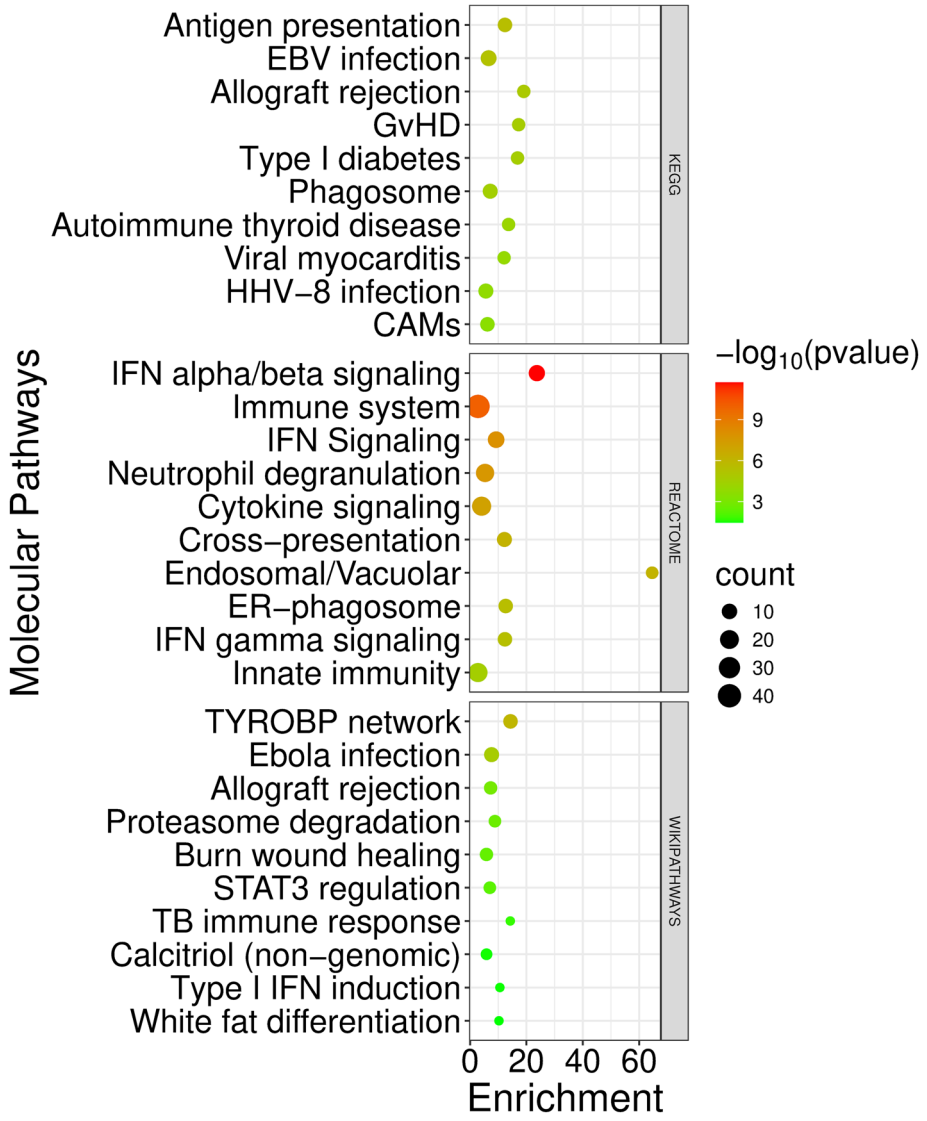

Figure 3. The top ten enriched metabolic pathways were closely related to cardiomyopathy enriched by DEGs between the low and high-risk groups in KEGG, WiKi, and Reactome databases.

Biomarkers (key hub proteins and TFs) validation

We validated biomarkers using the receiver operating characteristic (ROC) curve for the GEO profile dataset with access number GSE26887 for three key hub proteins (ITGAX, IRF1, and MX1) and four TFs (GATA2, FOXC1, SREBF1, and STAT3). The GEO profile dataset with access number GSE1869 for IRF7, and the GEO profile dataset with access number GSE3585 for HLA-B and NFKB1, as shown in Fig. 9. We found that the area under the ROC curve (AUC) for the hub proteins ranged from 0.716 to 0.884 and for the TFs from 0.632 to 0.925, indicating good validation performance. The overall information on ROC analysis results is described in Table 1.

Discussion

Cardiomyopathy is a highly dangerous condition that can take a person's life. Treatment for cardiomyopathy is extremely difficult because of its variety. Therefore, tailored treatment for cardiomyopathy will be beneficial, but the prognosis for people with cardiomyopathy is poorer[31]. Consequently, a crucial aim for advancing the personalized and precise treatment of cardiomyopathy in the future involves the creation of novel biomarkers utilizing heterogeneity and single-cell RNA sequencing (scRNA-seq) data. In this study, we employed bioinformatics methodologies to analyze single-cell RNA sequencing (scRNA-seq) and RNAseq data derived from the peripheral blood of patients with cardiomyopathy. Our objective was to explore the presence of cellular heterogeneity, identify differentially expressed genes (DEGs), research relevant biological pathways, construct a protein-protein interaction (PPI) network, identify key hub proteins, analyses of microRNAs (miRNAs), and assess transcription factors (TFs). Based on our raw dataset, the PBMCs clustered in 28 cellular subsets using the advanced tools of scRNA-seq. In our study, we observed consistent cell proportions for each cell type across the

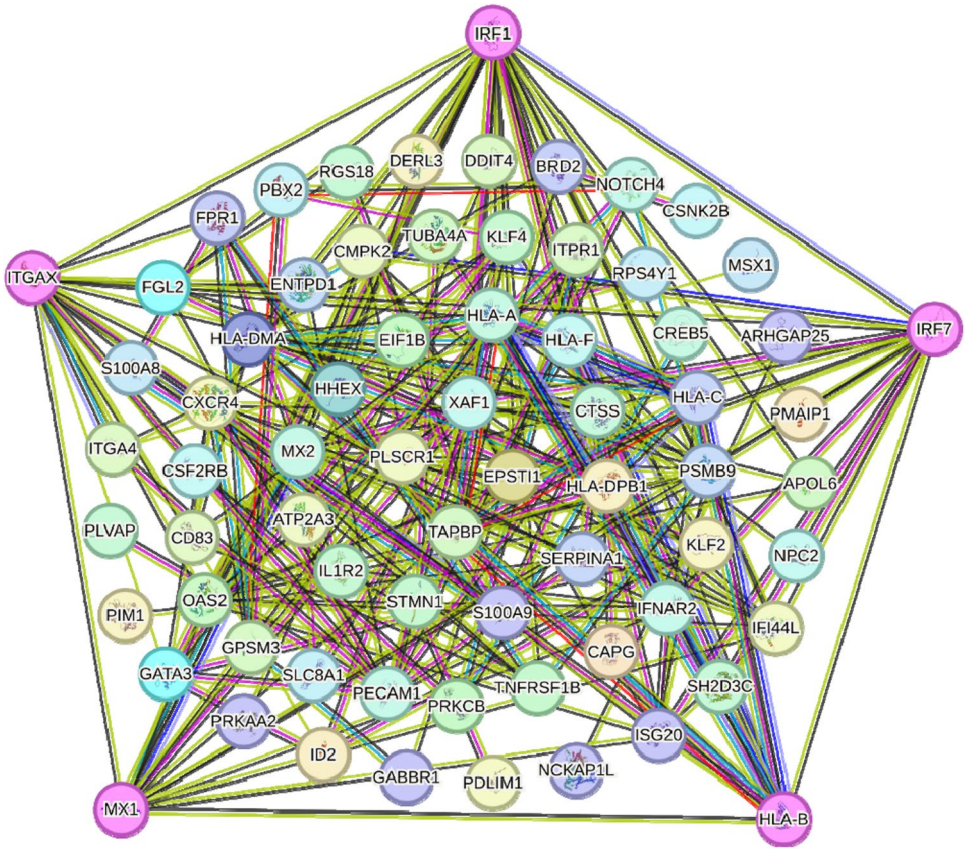

Figure 4. Visualization of the PPI network of DEGs that are shared by the cardiomyopathies was constructed using the STRING database. The nodes highlighted with pink color indicate the hub genes.

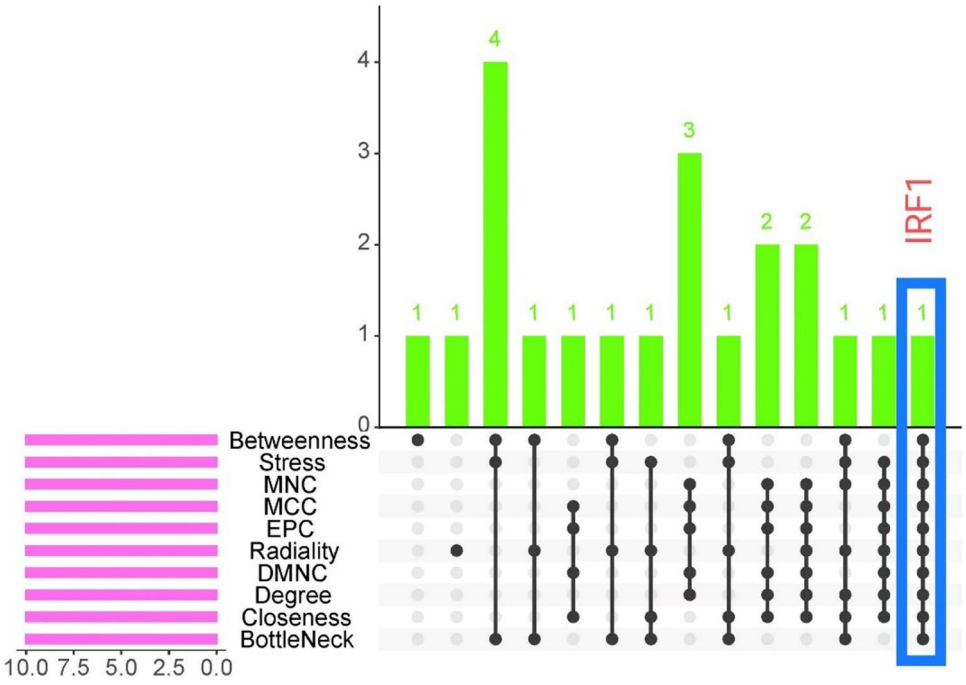

Figure 5. Upset plot of DEGs using 10 network methods from cytoHubba; where IRF1 has covered each of the methods.

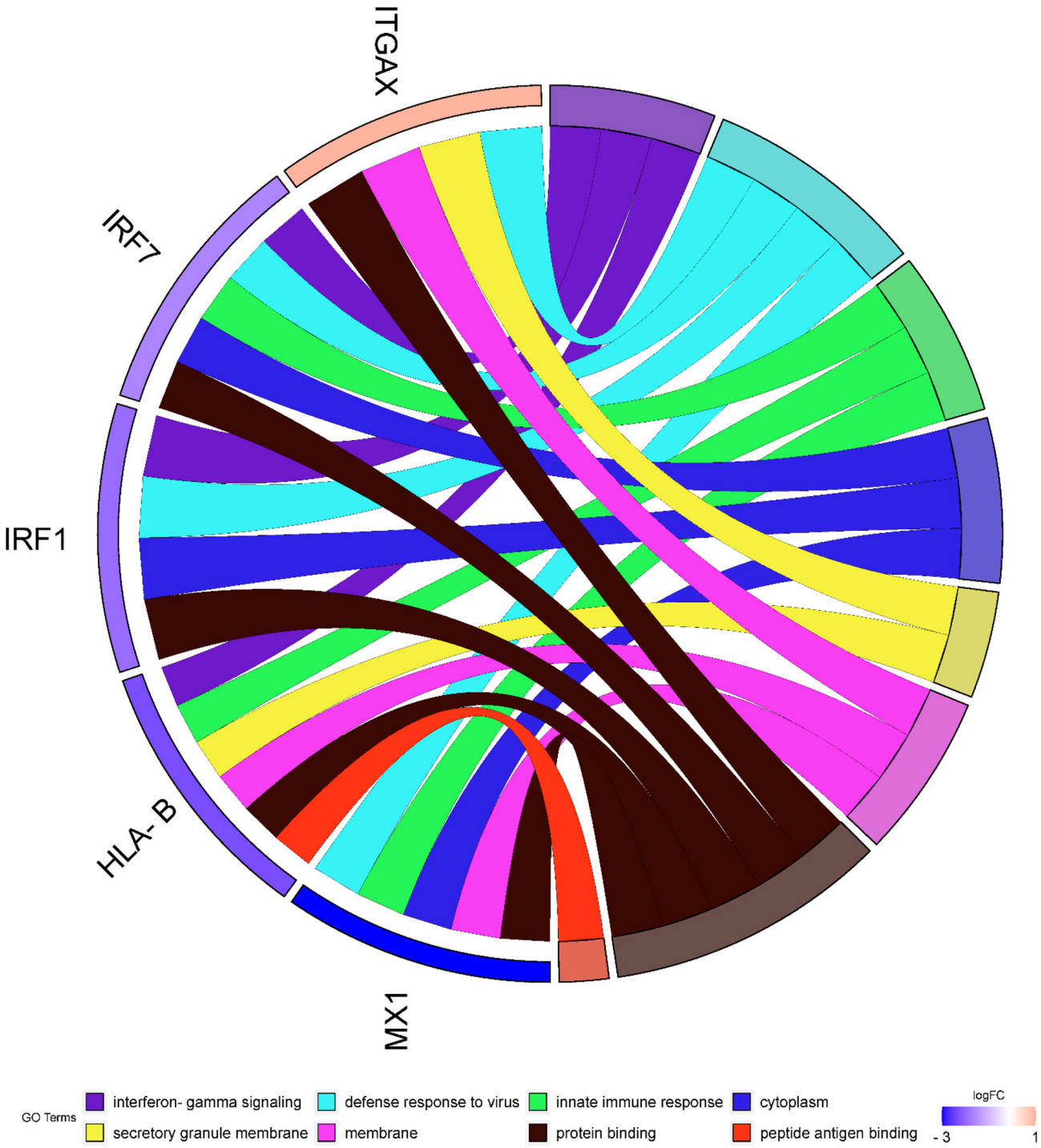

Figure 6. Correlation of key hub proteins with the most significant gene ontology terms by chord plot analysis of cardiomyopathy based on logFC value.

five experimental groups, namely natural killer cells, naïve B cells, T cells, monocytes, and dendritic cells. On the other hand, sequencing data are frequently used in biomedical research and have become a major resource for discovering biomarker candidates. A popular method for identifying DEGs in a variety of disorders, including cardiomyopathy of gene expression profiling[32]. The identification of specific DEGs helps highlight the molecular changes in cardiomyopathy. Many of these genes are involved in immune response and tissue damage repair, suggesting that inflammatory processes play a crucial role in disease progression. These findings support the hypothesis that cardiomyopathy may involve dysregulation of both immune and fibrotic pathways, offering potential biomarkers for disease progression and treatment[33]. Substantial changes in the expression profiles of 123 genes in datasets matched to the dendritic cell cluster were found by analyzing gene expression patterns in

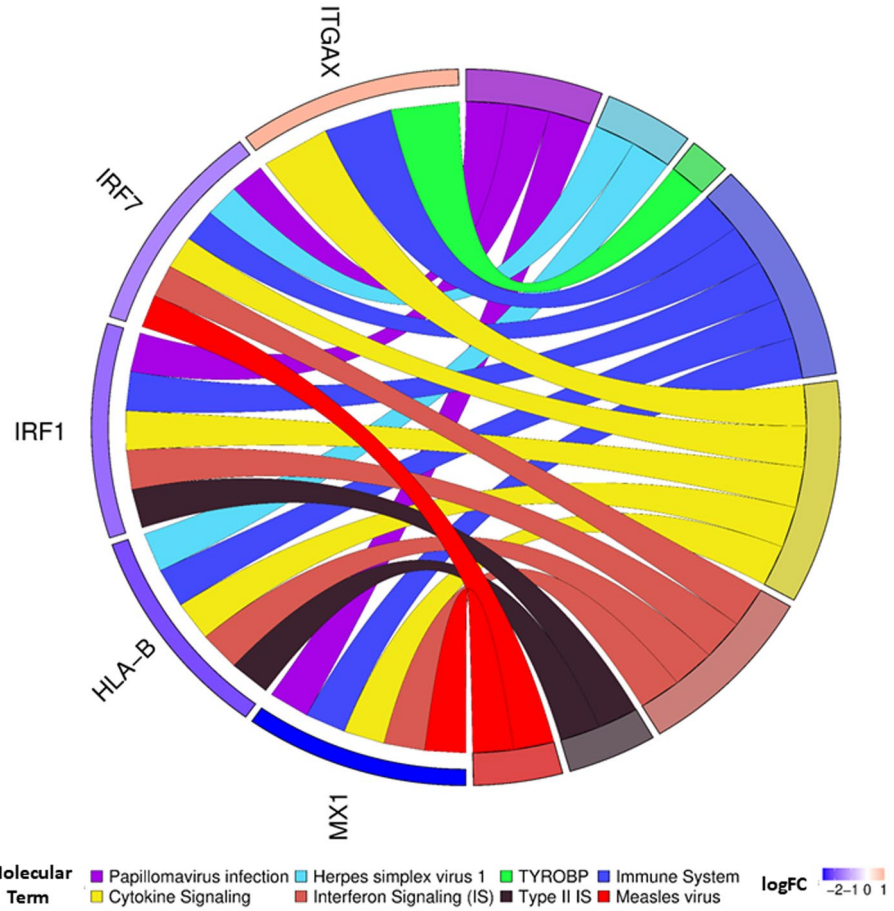

Figure 7. Correlation of key hub proteins with the most significant molecular terms by chord plot analysis of cardiomyopathy based on logFC value.

the peripheral blood monolayer cells (PBMC) of patients with cardiomyopathy. We worked with two datasets, GSE65446 and GSE155495 that shared differentially expressed genes with a dendritic cell cluster.

In the context of BP, CC, and MF, the gene ontology (GO) offers a comprehensive framework and a collection of conceptual tools to systematically characterize the functional activities carried out by gene products in many species[34]. Our investigation has shown that one of the key gene ontology concepts connected to biological processes is translation. This step in particular is in charge of starting the translation of RNA molecules related to certain biomolecules[35]. The most enriched terms are shown to have a good link with cardiomyopathy in the context of form BP. It has been discovered that there is a strong linkage between cardiomyopathy and signal transduction, cell communication, immune response, carbohydrate-mediated signaling, and cell surface receptor-linked signal transduction. It has been determined that the deficiency of this specific protein may be a contributing cause of cardiomyopathy [36,37]. Regarding CC, a lot of the current research on the subject is devoted to figuring out the processes that control the creation and targeting of channels as well as their complexes in the cardiac myocytes' plasma membrane[38]. Various cardiac disorders have been caused by lysosome malfunction[39]. The extracellular matrix network is vital for maintaining cardiac homeostasis because it transmits important signals to cardiomyocytes, vascular cells, and interstitial cells in addition to supporting structure and allowing force transmission[40]. Exosomes are essential for the remodeling of the cardiac muscle[41]. In the context of MF, in acute myocarditis, viral antigens are displayed on major histocompatibility antigen (MHC) class I molecules, and infected cells are destroyed by perforin that is produced by $CD8^+$ T lymphocytes[42]. A growing body of research over the past ten years suggests that changes in the excitation-contraction electrical current coupling may be a key factor in the pathophysiology of cardiac failure[43]. The extracellular matrix, growth factor binding, integrin binding, and PDGF binding were all impacted by decreased intercellular communication in hypertrophic cardiomyopathy (HCM), whereas adenylate cyclase binding, calcium channel inhibitor activity, and serine-threonine kinase activity were all elevated in non-obstructive HCM[44].

Conversely, we found significant pathways in WiKi, Reactome, and KEGG databases. Pathway studies illustrate the organism's response to changes in itself. Using underlying molecular or biological pathways analysis is a model-based method for illustrating how many illnesses interact with one another[45]. Regarding KEGG, there is insufficient evidence to determine the precise causes lately allograft failure in cardiac transplantation[46]. Dilated cardiomyopathy is frequently the result of autoimmune myocarditis. It is not known which antigen-presenting cell initiates autoimmunity, even though T cell sensitivity to cardiac self-antigen is frequently

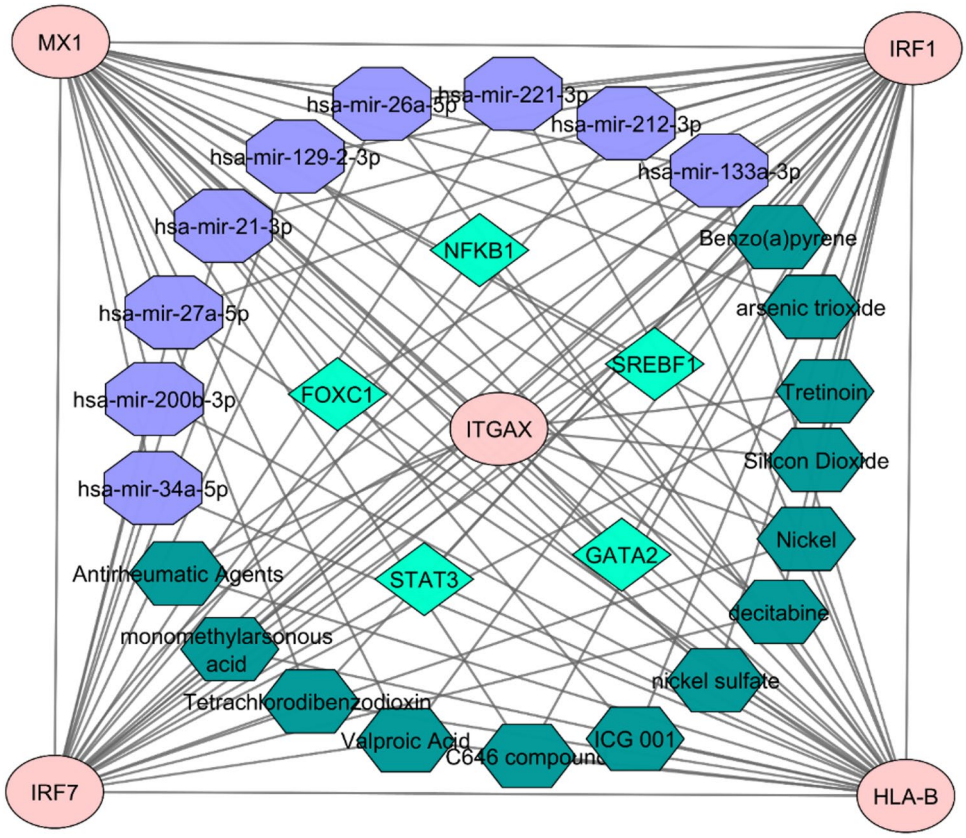

Figure 8. Interaction network for regulatory elements with key hub proteins. Here, miRNAs are indicated by octagon shape, chemicals by hexagon shape, TFs by diamond shape, and key hub proteins by ellipse shape with pink color.

observed in the illness[47]. Early detection and treatment of chronic active Epstein-Barr virus infection is critical due to chronic active myocarditis disarrays and reduces cardiomyocytes, which accelerates the development of heart failure[48]. According to Reactome, the pathophysiology of viral heart disease is similar to that of many other viral disorders in that it involves both direct viral harm and the host's immunological response[49]. By producing pro-inflammatory mediators, granular particles, and reactive oxygen species, neutrophils can exacerbate cardiac damage. According to more recent research, neutrophils can worsen inflammation and heart damage by producing extracellular vesicles and extracellular traps[50]. Within the context of WiKi pathways, cardiomyopathies, heart failure, myocardial ischemia, and hypertrophy all appear to be related to proteasome dysfunction[51]. Ad- Ebola virus glycoprotein (EGP)-transduced primary human cardiac microvascular endothelial cells demonstrated a lack of adhesion to the plastic surface, which was followed by cell death[52].

Furthermore, in the context of molecular pathways, we have found maximum pathways related to infectious agent pathways as well as other wound healing pathways. The presence of infectious agent-related pathways, such as those involving viral or bacterial responses, may initially appear unexpectedly in the context of a non-infectious disease. However, it is important to recognize that these pathways often share common signaling mechanisms with inflammatory and immune responses that are activated in non-infectious conditions, such as cardiomyopathy or tissue injury[53]. Specifically, many of the pathways related to infectious agents, such as Toll-like receptor (TLR) and cytokine signaling, are also critical components of the immune response to sterile inflammation. In the context of tissue damage or stress, such as in cardiomyopathy, these pathways are often upregulated as part of the body's attempt to repair and remodel the affected tissue. This immune activation is not specific to pathogens but can be triggered by damage-associated molecular patterns (DAMPs) released during cell injury, mimicking an infectious response[54,55].

On the other hand, the wound healing pathways identified, while traditionally associated with physical injury, are also relevant to tissue repair processes in chronic diseases. In cardiomyopathy, for instance, fibrosis and tissue remodeling are common as the heart attempts to heal itself following stress or damage. This often involves pathways related to extracellular matrix organization[56], angiogenesis[57], and immune cell recruitment[58], which overlap significantly with the wound healing processes observed in more classical injury models. Besides, it is well-established that immune pathways initially evolved to combat infections can also be co-opted during sterile inflammatory responses. For example, NOD-like receptor signaling[59] and NF-kB activation[60], typically seen in pathogen defense, are also prominent in cardiomyopathic conditions were inflammatory cytokine release drives disease progression. These findings suggest that both immune activation and tissue repair mechanisms are significantly altered, underscoring the potential role of chronic inflammation and aberrant tissue remodeling

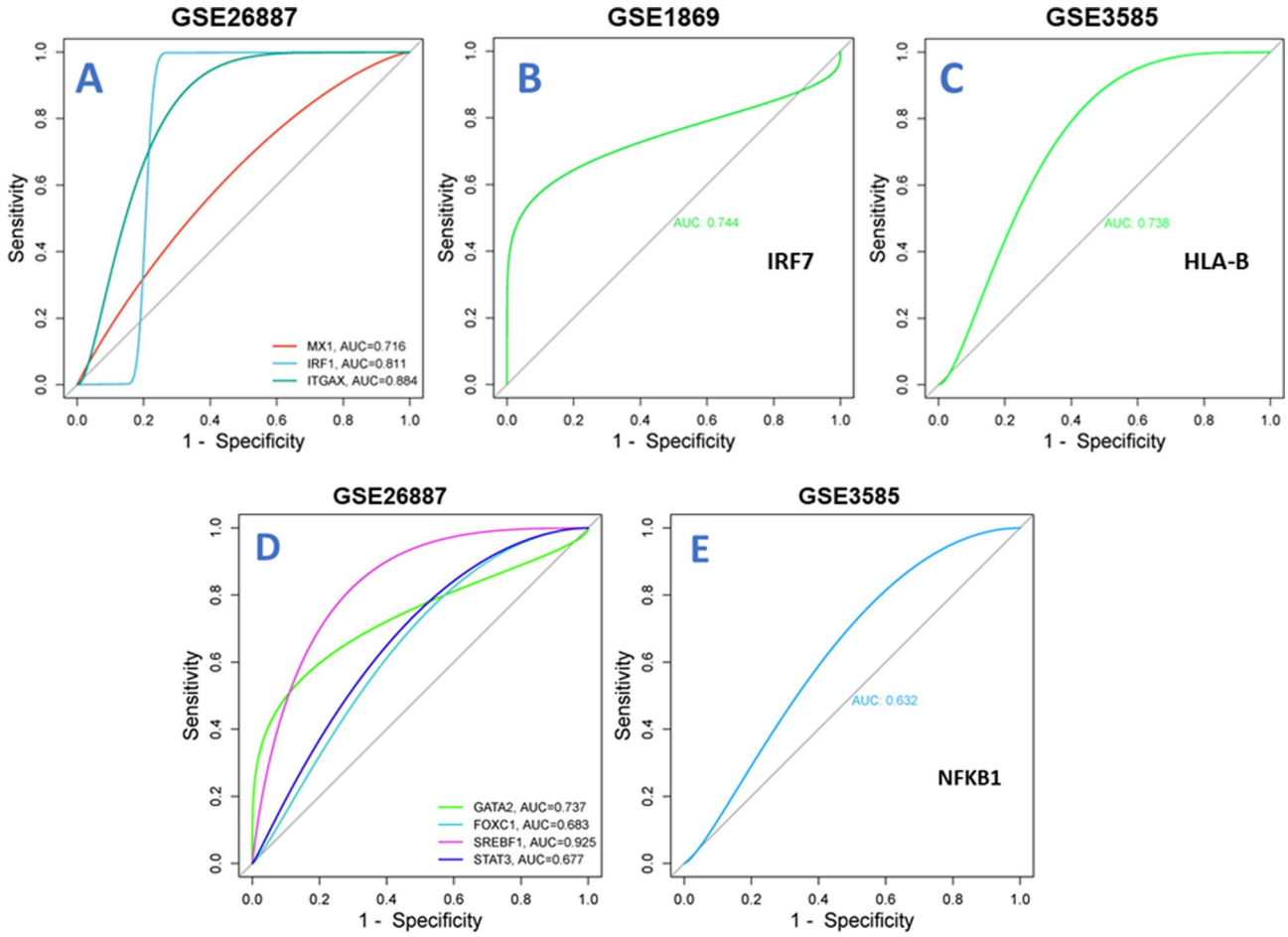

Figure 9. ROC curve analyzing the detection performance of biomarkers in cardiomyopathy. Hub proteins including (**A**) IRF1, MX1 and ITGAX; (**B**) IRF7; (**C**) HLA-B; and Transcription factors (TFs) including (**D**) GATA2, FOXC1, SREBF1 and STAT3; (**E**) NFKB1.

Biomarker Type	Biomarker Symbol	Description	UniProt ID	AUC	Dataset
Hub Proteins	IRF1	Interferon regulatory factor 1	P10914	0.811	GSE26887[28]
	MX1	MX Dynamin Like GTPase 1	P20591	0.716	
	ITGAX	Integrin Subunit Alpha X	P20702	0.884	
	IRF7	Interferon Regulatory Factor 7	Q92985	0.744	GSE1869[29]
	HLA-B	Major Histocompatibility Complex, Class I, B	P01889	0.738	GSE3585[30]
Transcription Factors	GATA2	GATA Binding Protein 2	P23769	0.737	GSE26887[28]
	FOXC1	Forkhead Box C1	Q12948	0.683	
	SREBF1	Sterol Regulatory Element Binding Transcription Factor 1	P36956	0.925	
	STAT3	Signal Transducer and Activator Of Transcription 3	P40763	0.677	
	NFKB1	Nuclear Factor Kappa B Subunit 1	P19838	0.632	GSE3585[30]

Table 1. Validation of biomarkers details with their ROC curve analysis.

in disease progression. This analysis links molecular alterations to functional consequences, guiding therapeutic strategies aimed at modulating these pathways. Based on the 10 approaches of cytoHubba algorithms, we found five key hub proteins (ITGAX, IRF1, IRF7, MX1, and HLA-B). Studies on cardiomyopathy and other cardiac muscle diseases have supported each of the five identified key hub proteins. The macrophage/dendritic cell marker ITGAX may potentially serve as an indication and be a possible biomarker for heart failure (HF) or heart muscle disease (cardiomyopathy)[61]. Previous research discovered that IRF1 and IRF7 have a unique function in controlling stress-induced cardiomyopathy [62,63]. The MX1 gene encodes the guanosine triphosphate (GTP) metabolic protein that has been implicated in the cellular antiviral response and MX1 as a cardiac disease predictive biomarker[64]. The gene biomarker HLA-B is a cause of heart muscle disorders[65]. ITGAX (CD11c)

identifies pro-inflammatory macrophages that stimulate inflammation, fibrosis, and myocardial damage, all of which are important facts of cardiomyopathy. In diseases like diabetic cardiomyopathy and heart failure, these macrophages release cytokines such as TNF-α, IL-1β, and IL-6, which cause detrimental tissue remodeling. By focusing on the ITGAX pathways, a therapeutic approach to enhance heart function may be possible by lowering detrimental macrophage activity, reducing inflammation, and preventing fibrosis[66–68]. Both IRF1 and IRF7 play a major role in controlling inflammatory responses in cardiomyopathy. Recent research indicates that IRF1 knockout decreases cardiac remodeling and that IRF1 regulates genes associated with fibrosis and inflammation, such as iNOS, which leads to heart hypertrophy and dysfunction. When overactivated, IRF7, which controls type I interferon responses, worsens chronic inflammation in the heart. It shows promise to treat cardiomyopathy by reducing inflammation and fibrosis through targeting both IRF1 and IRF7[69,70]. Recent studies have focused on MX1, a protein essential for the immune response against viral infections, in cardiovascular disorders such cardiomyopathy. It affects the dynamics of the mitochondria, which are essential for heart cell activity and preventing heart failure. Dysfunction of mitochondria is associated with oxidative stress and mitochondrial issues. By maintaining mitochondrial function and lowering heart stress, targeting MX1's mitochondrial GTPase activity may present novel therapeutic approaches for the treatment of cardiomyopathy[71]. Therefore, based on our research on cardiomyopathy, we may conclude that these three hub proteins (ITGAX, IRF1, and IRF7) are unique and have a strong predictive value for treatment. The utility of these markers in identifying and defining treatment for cardiomyopathy lies in their potential to guide immunomodulatory therapies. These proteins may serve as key intervention points for reducing inflammation-driven damage in the heart, making them valuable targets for further study.

The JASPAR database of NetworkAnalyst was used to build the key hub proteins and TFs interaction networks. As transcriptional regulatory factors, five TFs (GATA2, FOXC1, SREBF1, STAT3, and NFKB1) with degrees > 3 were chosen. The FOXC1 is one of the known TFs, is closely connected to other members, and plays a variety of vital functions in the development of the cardiovascular system, including cardiomyopathy is linked to mutations in human FOXC1[72]. GATA2 is linked to cardiomyopathy and suppresses the cardiac development of human mesoderm[73]. Nuclear Factor (NF) members include NFKB1, whose gene has also been linked to dilated cardiomyopathy[74]. Patients with end-stage heart failure have been linked to STAT3 down-regulation in the heart. Furthermore, a number of investigations have demonstrated that STAT3 activation enhances cardiac angiogenesis, cardiomyocyte survival, and hypertrophy in response to a variety of pathophysiologic stimuli, indicating a considerable benefit of STAT3 for the heart. The upregulation of STAT3 in our study may indicate a maladaptive immune response in cardiomyopathy, particularly driven by the type 1 IFN signaling cascade[75,76]. Given that STAT3 is down-regulated in failing hearts, pharmacological approaches aimed at improving heart function in the context of ischemia and the shift from compensated states to cardiac failure should be investigated[77]. Cardiomyopathy and sterol regulatory element-binding transcription factor 1 (SREBF1) are associated confirmed by Ambrosini et al.. studies[78]. In conclusion, while these markers and TFs are associated with general inflammatory and type 1 IFN pathways, their upregulation in the context of cardiomyopathy suggests a specific and potentially targetable immune signature. Targeted inhibition of type 1 IFN signaling has been explored as a therapeutic strategy in autoimmune and inflammatory diseases.

The interaction network was constructed to establish the relationship between the key hub proteins and miRNAs. Several microRNAs were selected as post-transcriptional regulatory factors for important hub proteins, based on a minimum degree score. Additionally, it has been claimed that hsa-mir-26a-5p is a risk factor and that it slows the development of several cardiac conditions, including cardiomyopathy[79]. Scientists demonstrated that the predictive biomarker for cardiomyopathy is hsa-miR-34a-5p[80]. It is thought that miR-21-3p is essential for the pathogenic processes and development of DCM[81]. Another research mentioned that one of the most significant predictive biomarkers for heart failure disorders, such as cardiomyopathy, is hsa-miR-129-2-3p[82]. The etiology of the cardiac muscle dysfunction can be attributed to the expression of hsa-mir-27a-5p[83]. AP Rubiś et al. conducted a study and concluded that cardiomyopathy and hsa-mir-133a-3p are independently correlated[84]. Changes in cardiovascular and cerebrovascular illness are also linked to hsa-mir-221-3p[85]. It has also been observed that cardiac tissues regulate hsa-mir-212-3p and hsa-mir-200b-3p[86,87].

Thirteen potential chemical compounds have been identified from protein-chemical compound interactions analysis. The tetrachlorodibenzodioxin, valproic acid, tretinoin and arsenic trioxide have been predicted to be linked to cardiomyopathy based on disease interactions because those chemical compounds were highly associated with cardiomyopathy[88]. Based on the findings of our literature review, a robust association has been observed between these microRNAs (miRNAs) and the development of drugs in individuals with cardiomyopathy treatment. In the final phase, we evaluated the diagnostic effects of our biomarkers with the help of ROC analysis. Our biomarkers (hub proteins and TFs) have shown good performance during analysis.

The results from these analyses collectively advance our understanding of the molecular mechanisms underlying cardiomyopathy. They point to the central role of immune dysregulation, particularly the involvement of type 1 interferon signaling and inflammation, in driving disease progression. Moreover, the identification of hub proteins and key transcription factors provides potential therapeutic targets that can be explored in future studies to develop more effective treatments for cardiomyopathy. Despite several limitations including wet lab experiments, we provide a thorough overview of the variations, which can serve as a resource to guide future studies aimed at identifying the most effective approach for managing or potentially curing the disease.

Conclusion

The identification of putative biomarkers and associated pathways will be necessary for the effective application of disease-modifying treatments in the advancement of cardiomyopathy. This study summarized network-based methods for determining the metabolic pathways that underlie the development of cardiomyopathy. About 123 DEGs were discovered from two transcriptome data sets of cardiomyopathy using a thorough bioinformatics

analysis to create a PPI network. The hub genes with the highest significance from the PPI network were then identified as potential new biomarkers in the diagnosis of cardiomyopathy. Subsequently, several TFs and miRNAs were discovered among the shared genes associated with cardiomyopathy. The ROC analysis demonstrated the biomarkers' diagnostic efficacy. According to our predictions, each of these biomarkers will make it possible to identify cardiomyopathy more quickly and affordably by detecting cardiac samples. Our atlas offers a valuable framework for enhancing our understanding of this intricate immune-mediated disease. Consequently, we propose a more comprehensive validation of this paradigm as well as the potential biomarker transcripts identified by our clinical research.

Materials and methods

Data collection and preprocessing

The scRNA-seq data of PBMCs, which are accessible to the public, were obtained from the Broad Institute's website (PMID: 28212749)[89]. The Seurat package 4.3.0 version 4.3.1 of R[90] was used to process the data for quality evaluation, normalization and further analysis. We proceed to calculate the count of features within the sample that demonstrate significant variation among cells, indicating a high level of expression in certain cells while being low in others[91]. Three cells and a minimum of 200 features with nonzero counts were needed for the gene retrieval process to provide quality control[90]. Normalizing the data is important to account for and minimize cell-specific bias, which may affect further uses such as the detection of differential gene expression[92]. Normalization is used to account for measurement errors resulting from unfavourable biological effects in samples and characteristics (genes)[93].

Clustering is a valuable strategy for locating groups of cells with similar gene expression patterns. First, cells are systematically grouped together using the Louvian algorithm, a graph-based clustering approach based on K-nearest neighbor (KNN). The maximum standard modularity function is the aim of cluster analysis[94]. The information from Seurat data is first converted to the format needed for the reference-based cluster annotation, which is implemented in a different module called SingleR[94]. We further employed dimensionality reduction techniques, such as Uniform Manifold Approximation and Projection (UMAP), to visualize the clusters and verify whether the resulting separations corresponded to known dendritic cell subtypes or appeared as artificial splits[95]. Lastly, side-by-side comparison of filtered data made by R's Monaco package using the MonacoImmune Data function is made possible by annotation based on external references[25]. Additionally, differential gene expression analysis between clusters was performed to ensure that each identified cluster exhibited unique transcriptional signatures consistent with distinct biological populations.

On the other hand, two RNAseq datasets, GSE65446 and GSE155495 obtained from the GREIN, were utilized in this study to evaluate the diagnostic efficacy of the discovered DEGs in cardiomyopathy. GREIN provides a user-friendly platform for performing complex differential gene expression analyses and gaining insights from large-scale transcriptomic data[96]. The GSE65446 dataset provides 6 cardiomyopathy samples and 4 normal samples while the GSE155495 dataset provides 6 cases and 6 normal samples. Figure 10 demonstrates the thorough strategy of combining systems biology and computational technologies to discover molecular markers and pathways in heart tissues impacted by Cardiomyopathy.

Statistical analysis of datasets and identification of differentially expressed genes

The primary goal of this analysis was to identify genes whose expression is significantly altered between control and cardiomyopathy samples, aiming to uncover key molecular pathways involved in the pathogenesis of cardiomyopathy. This analysis serves as the foundation for identifying candidate biomarkers and therapeutic targets[97].

We were applied a threshold of adjusted p-value < 0.05 and log2 fold change > |1.0| for the screening of DEGs in both RNAseq and scRNA-seq datasets. The adjusted p-value (Benjamini-Hochberg method) was used to control the false discovery rate (FDR) due to multiple testing. This threshold is widely accepted in single-cell and bulk RNA sequencing studies to ensure a balance between sensitivity and specificity, minimizing false positives while identifying biologically meaningful changes [98,99]. The shared DEGs among the GSE65446, GSE155495 and scRNA-seq datasets targeted cell were discovered by the web-based application Venny 2.1.0 [100].

Functional enrichment and pathway analysis of DEGs

The goal of this analysis was to place the differentially expressed genes (DEGs) within a functional context by identifying enriched biological pathways. Pathway and gene ontology (GO) were employed to provide a fantasy module for prediction and to interpret the enrichment analysis of bio-term categorization processes and gene clusters[101]. For the gene ontology and functional enrichment of shared DEGs, we employed the software application Funrich (version 3.4.1)[102]. We generated pathways for WiKi, Reactome, and KEGG[103] of shared DEGs via the functional enrichment tool DAVID[104] and the web application SRplot[105]. To identify significant functional and pathway terms, we took into reference the P-value < 0.05 criteria. This threshold is consistent with widely accepted standards in the literature for identifying biologically relevant pathways[106].

Protein-protein interaction (PPI) and hub proteins identification

PPI analysis is an effective method for figuring out how the cell functions, comprehending disease processes, finding possible therapeutic targets, and learning about the structure and function of proteins[107]. The PPI network of shared DEGs in this investigation was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING v11.0) database[108]. Cytoscape (version 3.7.1)[109] was utilized as the tool for determining the protein-protein interaction networks among the shared differentially expressed genes (DEGs). The aim of this analysis was to identify central regulatory proteins within the protein-protein interaction (PPI) network that may serve as key drivers of the observed molecular alterations. By pinpointing these hub proteins, we can prioritize targets

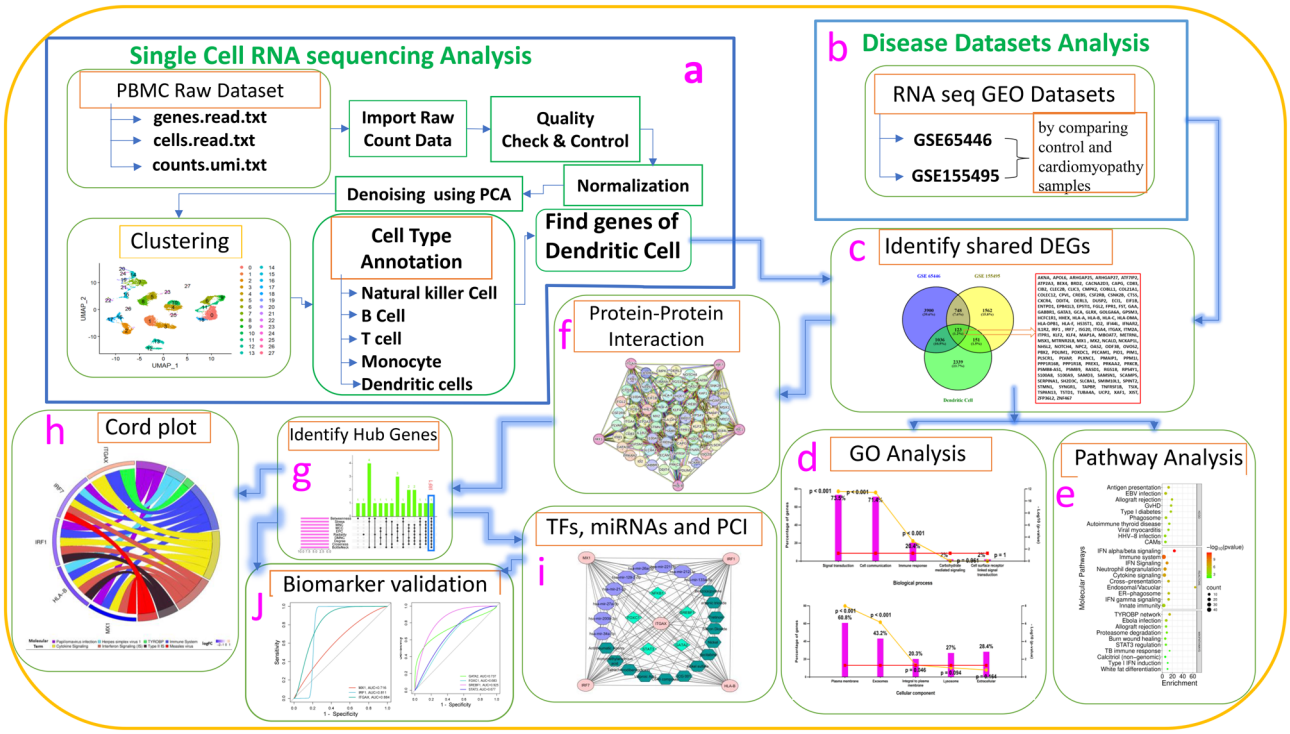

Figure 10. A schematic flowchart represents the workflow to identify dendritic cell specific novel biomarkers and biological pathways in cardiomyopathy through a series of computational analysis.

for future mechanistic studies or therapeutic interventions[110]. In our study, we identify key hub proteins using ten topological methods: Betweenness, Stress, Maximum Neighborhood Component (MNC), Maximum Clique Centrality (MCC), Edge Percolated Component (EPC), Radiality, Density of Maximum Neighborhood Component (DMNC), Degree, Closeness, and BottleNeck in the cytoHubba plugin of Cytoscape[111]. Hub proteins were defined as those with a degree greater than the 75th percentile of the degree distribution. This approach is frequently employed in network biology to identify highly connected proteins that may serve as key regulators[112]. We have also created an Upset plot in the online tool SRplot to visualize the key hub proteins that are used in ten methods of cytoHubba.

Co-relation between pathways and key hub proteins

The relationship between the enriched Gene Ontology (GO) categories or molecular pathways that correspond to the logFC values of key hub proteins is graphically shown by the chord plot. The use of a chord plot to analyze the correlation between pathways and hub proteins provides a clear, visual representation of how key proteins are central to multiple biological processes, helping in understanding their roles and potential as therapeutic targets[113]. The chord plot was created using SRplot[105], a free online tool. It is possible to determine the logarithm of fold changes (logFC) by analyzing differentially expressed genes (DEGs). We were applied a threshold of adjusted p-value < 0.05.

Regulatory interaction network analysis of key hub proteins

The goal of this analysis was to identify transcriptional regulators driving the differential expression of genes in cardiomyopathy. This helps elucidate the upstream regulatory mechanisms controlling the disease's molecular profile and provides additional targets for therapeutic intervention, particularly in modulating the immune and inflammatory responses[114]. Using the web tool NetworkAnalyst 3.0 [115], we implemented a regulatory interaction network among miRNAs and TFs of key hub proteins. This tool for bioinformatics interprets and displays data in the network configuration association. The miRNA and transcription factor (TFs) interaction networks were constructed using the TarBase and Jaspar databases, respectively via NetworkAnalyst. In the transcription factor analysis, we applied a regulatory significance score cutoff (e.g., z-score > 2) to select transcription factors significantly enriched in regulating DEGs. This threshold is based on statistical analysis of the regulatory interactions, and similar cutoffs have been applied in other studies of gene regulatory networks[116].

Analysis of protein–chemical compounds

To ascertain which particular chemical molecules are involved in the interaction between proteins in comorbidities, protein-chemical compounds can be examined. Protein-chemical compounds significance analysis evaluating binding affinities, predicting and validating interactions, and investigating biological importance[117]. After analyzing the key hub proteins using the Comparative Toxicogenomics Database (CTD)[118], we used the degree > = 3 as cut-off in NetworkAnalyst to identify protein-chemical interactions.

Biomarkers validation by ROC curve analysis

We performed the receiver operating characteristic (ROC) curve[119] for validation of key hub proteins and potential significant TFs[120]. The ROC curve is employed to identify the acceptable cut-off, which affects the test's sensitivity and specificity. The ROC curve provides a graphical representation of the true positive rate (sensitivity) versus the false positive rate (1-specificity) for different threshold values. ROC curve analysis is a key tool for validating biomarkers by assessing their accuracy, discriminative power, and potential clinical applicability[121]. The area under the curve (AUC) values ranged from 0.5 to 1.0, with bigger AUC values indicating better categorization[122]. We have collected GEO profile datasets from NCBI[123] and used SRplot[105] for visualization.

Data availability

PBMC data for scRNA-seq analysis was collected from the Single Cell portal. The Gene Expression Omnibus (GEO) database from GREIN was used to access the GSE65446 and GSE155495 datasets and GEO profile datasets from NCBI was used to access the GSE26887, GSE1869 and GSE3585 datasets. Data produced in this research can be available on request to the corresponding author (Dr. Md Habibur Rahman).

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