Utilising bioinformatics and systems biology methods to uncover the impact of dermatomyositis on interstitial lung disease

R. Ding[1], D. Liang[1], S. Huang[1], X. Huang[1], B. Wei[1], S. Wan[2], H. Zhang[2], Z. Wan[2]

[1]Department of Rheumatology and Immunology, Zhongshan Hospital Xiamen University, School of Medicine, Xiamen University, Xiamen, Fujian;
[2]Department of Oncology and Vascular Interventional Radiology, Zhongshan Hospital Xiamen University, School of Medicine, Xiamen University, Xiamen, Fujian, China.

Abstract

Objective

Dermatomyositis (DM) is frequently associated with interstitial lung disease (ILD); however, the molecular mechanisms underlying this association remain unclear. This study aimed to employ bioinformatics approaches to identify potential molecular mechanisms linking DM and ILD.

Methods

GSE46239 and GSE47162 were analysed to identify common differentially expressed genes (DEGs). These DEGs underwent Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis. A protein-protein interaction (PPI) network was constructed to identify hub genes and transcriptional regulators. Potential therapeutic drugs were predicted using the Drug-Gene Interaction Database (DGIDB).

Results

A total of 122 common DEGs were identified between the DM and ILD datasets. These DEGs were significantly enriched in signal transduction, transcriptional regulation, inflammation, and cell proliferation. Key pathways included the NOD-like receptor signalling pathway, cytokine-cytokine receptor interaction, and TNF signalling pathway. PPI network analysis revealed the top 10 hub genes: CD163, GZMB, IRF4, CCR7, MMP9, AIF1, CXCL10, CCL5, IRF8, and NLRP3. Additionally, interactions between hub genes and transcription factors/miRNAs were constructed. Eleven drugs targeting four hub genes (CXCL10, MMP9, GZMB, and NLRP3) were predicted using the DGIDB.

Conclusion

In summary, the study identified 10 key genes involved in the molecular pathogenesis of DM and ILD. Moreover, 11 potential drugs were identified that may offer viable therapeutic options for treating DM and ILD in the future.

Key words

dermatomyositis, interstitial lung disease, bioinformatics, different expression genes, targeted drugs

Rui Ding, MD
Di Liang, MD
Shimei Huang, MD
Xiaojing Huang, MD
Bo Wei, MD
Sirui Wan, MD
Hongjian Zhang, MD
Zheng Wan, PhD

Please address correspondence to:
Hongjian Zhang
Department of Oncology and Vascular Interventional Radiology, Zhongshan Hospital Xiamen University, 361000 Xiamen, Fujian, China.
E-mail: zhanghongjian11021@163.com

and to:
Zheng Wan
E-mail: wanzheng0626@xmu.edu.cn

Received on November 9, 2024; accepted in revised form on December 16, 2024.

Funding: this study was supported by Natural Science Foundation of Fujian Province (grant no. .2022J05298) and the Xiamen Medical and Health Guidance Project, China (grant no. 3502Z20244ZD1057).

Competing interests: none declared.

Introduction

Dermatomyositis (DM) is an autoimmune disorder (1) that affects both the skin and muscles (2), typically resulting in symmetrical muscle weakness and skin rashes (3). While DM diagnostic methods (4) include electrophysiological testing, skin biopsy, and lab tests, their limitations are well documented (5). For example, electrophysiological testing may lead to misdiagnosis as muscle inflammation may be mistaken for muscle weakness, which can also be caused by other factors (6). Additionally, skin biopsy may yield false-negative results, especially when atypical skin lesions are present. Furthermore, laboratory tests may not be conclusive since they cannot determine the specificity of the disease (7). Therefore, novel diagnostic approaches are necessary to improve DM diagnosis precision.

Approximately 60% of those with DM experience interstitial lung disease (ILD), the most frequent respiratory issue associated with the disorder (8). ILD is an array of diseases distinguished by inflammation and/or scarring in the lungs, leading to common symptoms such as shortness of breath (9), and low oxygen levels (10). The current ILD diagnostic methods are not effective (11). While high-resolution computed tomography (HRCT) of the chest is the most sensitive and specific imaging modality for ILD, it has drawbacks such as radiation exposure and potential false-positive results (12). Pulmonary function tests can provide valuable information, but they have limited sensitivity and specificity for ILD diagnosis (13). Lung biopsy is the gold standard for definitive diagnosis, but it carries a risk of complications and may not be feasible for all patients (14). Furthermore, the incidence and mortality rates of ILD vary based on the underlying cause. In idiopathic interstitial pneumonias, the most common type of ILD, the incidence ranges from 7 to 20 cases per 100,000 people per year, with a 40% mortality rate at 5 years (14). Overall, the correlation between DM and ILD highlights the importance of careful monitoring and prompt, accurate diagnosis to guide appropriate treatment.

To date, the pathogenesis of DM associated with ILD remains poorly understood, and effective diagnostic and therapeutic approaches for both conditions are yet to be established. Identifying shared molecular mechanisms between these diseases is essential, as it may provide a deeper understanding of their underlying pathogenesis and reveal novel therapeutic targets. Specifically, uncovering common pathways could illuminate key biological processes involved in both diseases, offering valuable insights for the development of targeted therapeutic strategies. Consequently, we employed bioinformatics and systems biology approaches to explore the molecular relationship between DM and ILD, delineate their molecular interactions, and identify potential pharmacological agents that may benefit both conditions.

Methods and materials

The workflow of this study

To investigate the potential relationship between DM and ILD, we conducted a bioinformatics analysis using two datasets: GSE47162, which includes samples from ILD patients, and GSE46239, which contains data from DM patients, both sourced from the Gene Expression Omnibus (GEO) database. We identified overlapping differentially expressed genes (DEGs) and performed pathway and enrichment analyses to explore the common biological functions between these two conditions. By revealing shared pathways, we aimed to enhance our understanding of the molecular interactions underlying both diseases, which could inform the development of new therapeutic strategies. Using the DEGs, we built a network to identify key hub genes and their associated transcriptional regulators, and further predicted potential drugs that may target both DM and ILD.

Datasets used in the study

Two microarray datasets were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/): GSE46239, which includes skin samples from 48 DM patients and 4 healthy controls, and GSE47162, which includes skin

samples from 23 ILD patients and 36 healthy controls. The GPL570 platform was used for the DM dataset, while the GPL10588 platform was used for the ILD dataset. The inclusion of these specific sample groups enables us to compare the gene expression profiles of both diseases in skin tissue, offering insights into potential shared molecular mechanisms and biological pathways.

Differentially expressed genes (DEGs)screened

GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) is a web-based tool provided by the GEO that enables differential gene expression analysis using datasets from microarray and RNA-Seq platforms. It allows users to compare gene expression levels across multiple experimental groups and identify DEGs. GEO2R employs statistical methods which applies t-tests and moderated t-statistics to assess the significance of gene expression changes. In this study, GEO2R was used to analyse the GSE46239 (DM) and GSE47162 (ILD) datasets, with DEGs selected based on an adjusted p-value threshold of <0.05 and log-fold change (logFC) cut-offs of >0.5 for upregulation and < -0.5 for downregulation.

The VennDiagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) is commonly used to generate Venn diagrams, which visually represent the overlap between multiple datasets.

In this study, it was used to visualise the overlapping DEGs between the DM (GSE46239) and ILD (GSE47162) datasets.

Functional and pathway enrichment analysis

By utilising gene ontology (GO) (15) and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis (16), the biological processes and pathways were deciphered. This investigation incorporated the DAVID database (https://david.ncifcrf.gov/) (17). The functional enrichment analysis considered three components – biological process (BP), cellular components (CC) and molecular function (MF) – and only accepted results with adjusted *p*-value of less than 0.05.

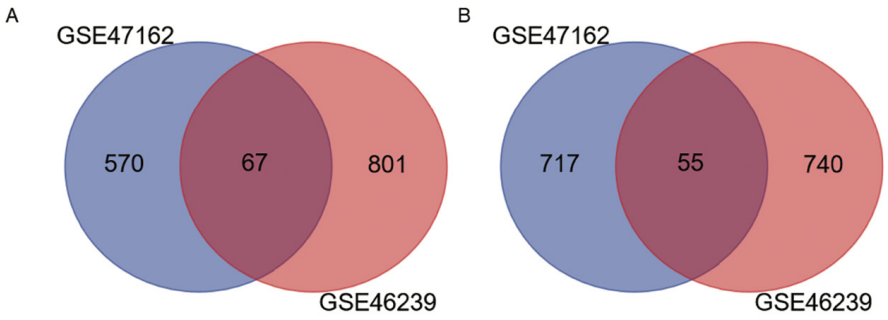

Fig. 1. Common differentially expressed genes (DEGs) identification. This study incorporates two microarrays comprising DM (GSE46239) and ILD(GSE47162). (**A**) 67 common DEGs with up-regulation between DM and ILD. (**B**) 55 common DEGs with down-regulation between DM and ILD.

Protein-protein interaction network (PPI) analysis and hub gene extraction

The Cytoscape soft version 3.9.0 was utilised to view and examine the PPI network. The CytoHubba plugin (18) was to identify the top 10 hub genes.

Recognition of transcription factors (TFs) and miRNAs involved with hub genes

TFs are bind to specific genes and regulate the transcription rate of genetic information, providing crucial molecular insights. The Network Analyst platform was used to identify topology-related TFs and miRNAs from the JASPAR database (http://jaspar.genereg.net) (19) and establish links between these TFs and our hub genes.

Prediction of potential drugs interacting with hub genes

Interactions between hub genes and drug molecules were examined using the drug database DGIDB (https://dgidb.genome.wustl.edu/) (20) to predict potential drugs for both DM and ILD.

Statistical analysis

The identification of DEGs in this study was performed using a two-sample t-test to compare the expression levels between the experimental groups (DM patients, ILD patients, and healthy controls). The t-test assesses whether there are statistically significant differences in gene expression between the groups. For each gene, the statistical significance was determined by adjusting the *p*-values for multiple comparisons using the Benjamini-Hochberg method to control the false discovery rate (FDR). A *p*-value threshold of less than 0.05 was considered significant, indicating that the gene expression difference was unlikely to have occurred by chance. To ensure that only biologically relevant changes were considered, we applied additional filtering criteria: log-fold change (logFC) values greater than 0.5 for upregulation and less than -0.5 for downregulation. This approach allowed us to identify DEGs that exhibited both statistical significance and meaningful expression changes, ensuring the robustness and relevance of the results.

Results

Identification of overlapping DEGs in DM and ILD

In our study, a total of 1663 DEGs were identified in the DM dataset, including 868 up-regulated genes and 795 down-regulated genes. Similarly, in the ILD dataset, we detected 1409 DEGs, of which 637 were up-regulated and 772 were down-regulated. We identified a set of 122 common DEGs (67 up-regulated and 55 down-regulated) shared between DM and ILD datasets. The cross-comparison analysis results are presented in Figure 1, and the list of these 122 DEGs is provided in Table I.

GO and pathway enrichment analysis

Several DEGs were seen to be in higher concentrations in BP that involve signal transduction, transcriptional regulation, inflammation, and cell proliferation. Additionally, the DEGs are primarily localised in the cytoplasm, cytosol, plasma membrane, and extracellular regions. The enriched MF of the DEGs include protein binding,

Table I. Differentially expressed genes (DEGs) identification.

DEGs	Gene name
Up-regulated	*RBP5; IGF1; NHLRC3; PLEK; NLRP3; RAB8B; ADAMDEC1; GMFG; PIK3AP1; SNX10; LILRA6; PARP14; MTHFD2; KIAA0226L; ACKR4; SELP; WARS; NOX4; VWF; APOL1; CDH11; GREM1; AIF1; KIF5C; GBP4; IL18RAP; CCR7; RGS1; TCEAL9; LYPD1; SLC38A6; GNLY; CCDC102B; CD163; GIMAP2; TNC; AFAP1L2; FAM198B; TAP2; CLEC7A; TNFSF13B; MDK; GZMB; PRSS23; ANKRD29; MFAP2; DLGAP5; CXCL10; GBP3; STAT2; CCL5; TM4SF1; TGFBR2; LINC00968; PPP2R3C; SMA4; APOBEC3G; MLKL; AIM2; IRF8; TDO2; CHN1; FMNL2; GGT5; C1orf162; FAM49B; MMP9*
Down-regulated	*GAPDHS; C1orf106; EP300; CAPN3; NDUFS1; GPR143; CLN8; ALDH3A2; CAMSAP3; TMEM159; BCL2L10; SLCO4A1-AS1; SLC6A15; RYR1; ZNF395; TFAP2B; SIRT2; DSTYK; DCT; FAM69C; KLC3; SNCA; AIF1L; OSBPL6; ARID1B; TBC1D10A; DPP6; ARHGEF5; MRPL43; IRF4; L1CAM; MKNK2; URGCP; GATA3; CCDC125; SENP2; STXBP6; ALDH3A1; PAMR1; COG5; IFNLR1; LGI3; APCDD1; TYR; KIF1A; PLLP; MLANA; MBP; TCP11L2; RRAGD; WNT4; AGAP1; ZNF703; CYB561A3; OCA2*

GTP binding, oxidoreductase activity, and calmodulin binding.

The KEGG pathway analysis identified several significantly enriched pathways related to NOD-like receptor signalling pathway, cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, influenza A, TNF signalling pathway, melanogenesis, and tyrosine metabolism. The pathways involved are crucial for the development of various illnesses, such as diabetes mellitus and interstitial lung disease. Therefore, the identified DEGs and their associated pathways may provide potential therapeutic targets for these diseases (Fig. 2).

PPI and hub gene extraction

Figure 3A depicts the PPI network for mutual DEGs of DM and ILD. The mutual DEGs-based PPI network had 72 nodes with 316 edges. Top 10 hub genes were CD163, GZMB, IRF4, CCR7, MMP9, AIF1, CXCL10, CCL5, IRF8, and NLRP3 (Fig. 3B). These genes are likely to represent potential therapeutic targets or biomarkers for these diseases.

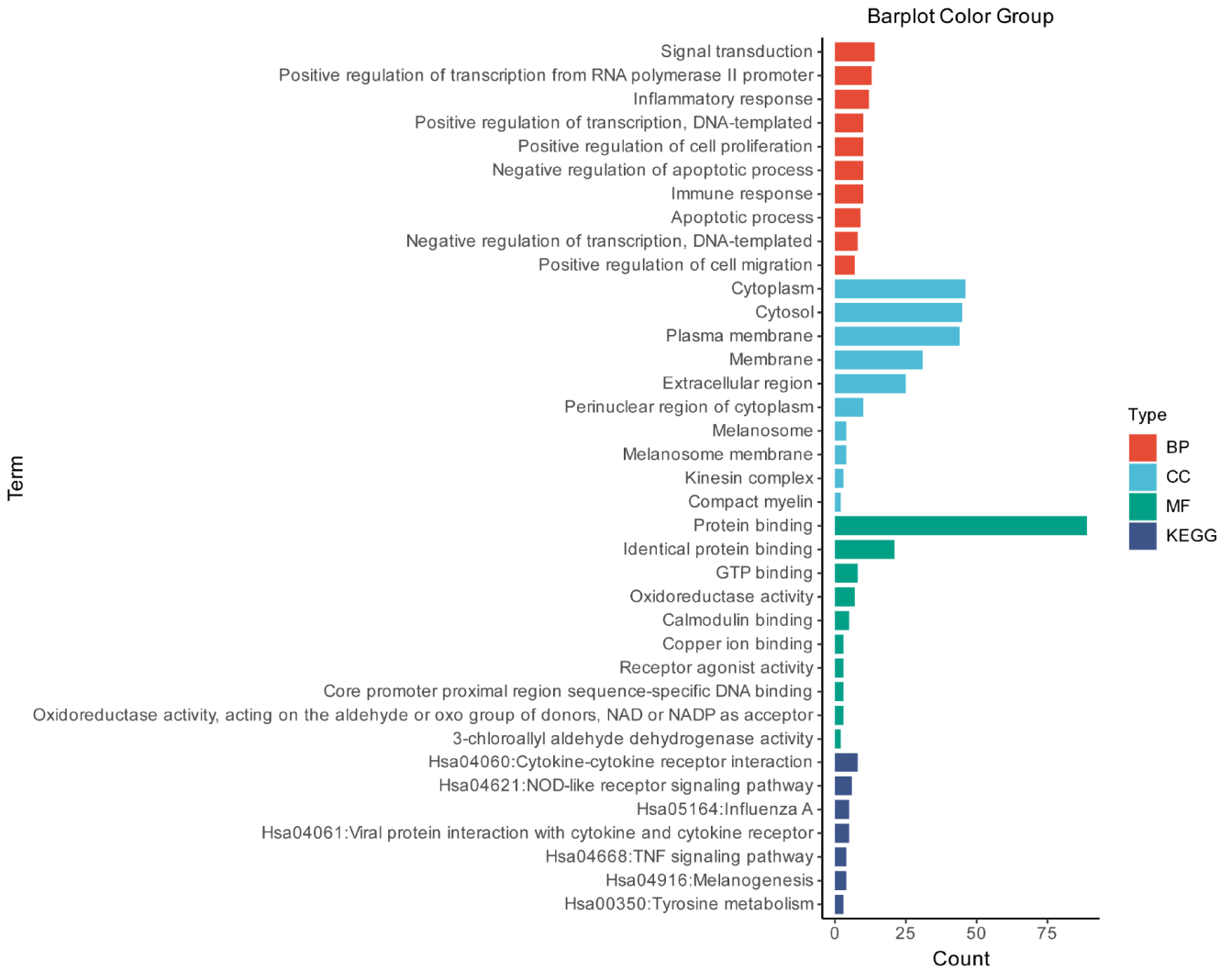

Fig. 2. Functional analyses of differentially expressed genes (DEGs) between DM and ILD. Red, blue, green, and dark indicating biological process (BP), molecular function (MF), and cellular component (CC), and KEGG pathway analyses, separately.

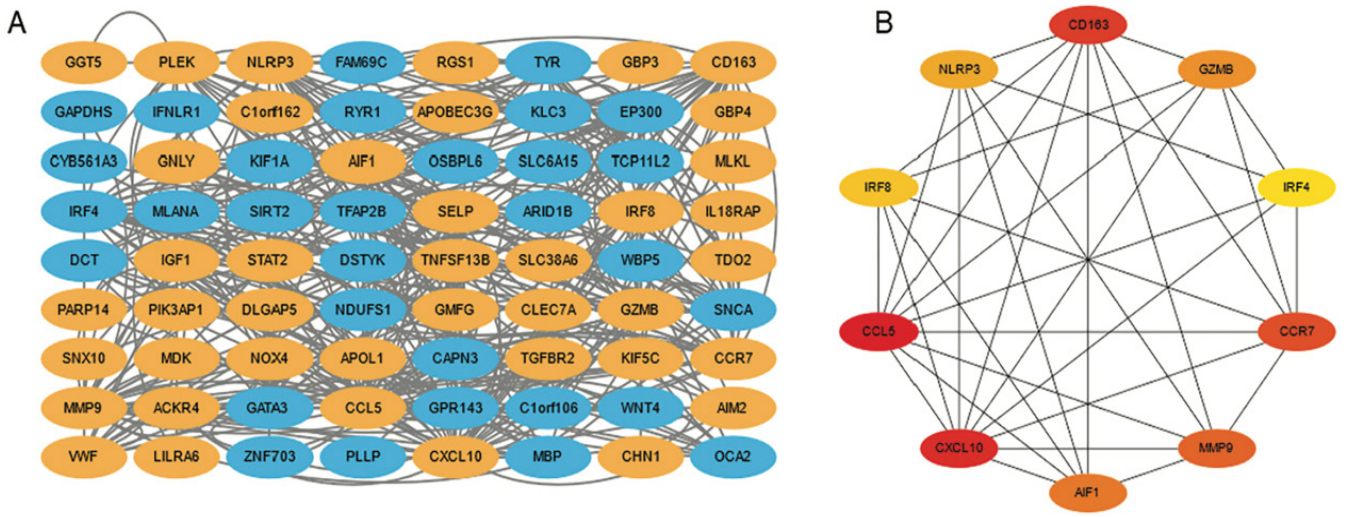

Fig. 3. Protein-protein interaction network (**A**) and hub genes (**B**). Yellow ellipses stand for up-regulation gene, while blue ellipses for down-regulation.

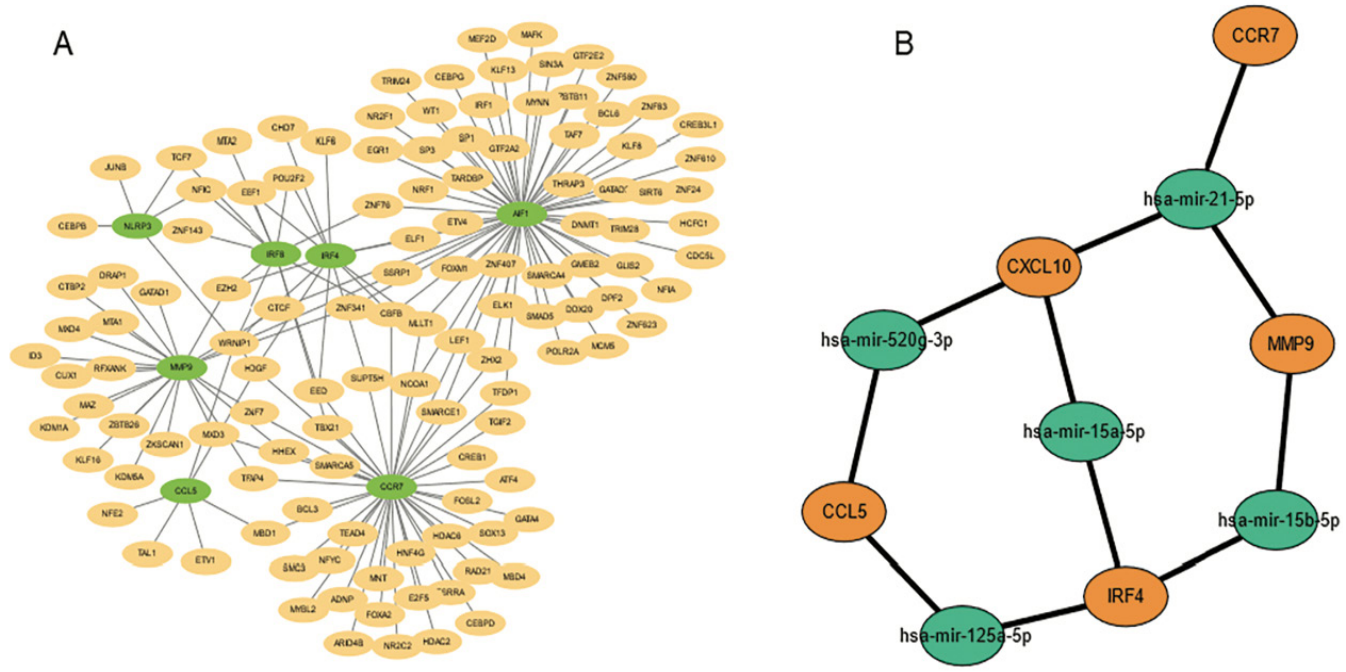

Fig. 4. Construction of regulatory signatures. (**A**) The hub genes and transcription factors (TFs) interaction network. Green ellipses represent hub genes, while yellow represents TFs. (**B**) miRNAs interactions with hub genes. Green represents miRNAs, brown indicates hub genes.
up-regulation gene, while blue ellipses for down-regulation.

Construction of regulatory signatures

Figure 4A illustrates seven hub genes (NLRP3, IRF4, IRF8, ATF1, MMP9, CCR7, and CCL5) targeted to 122 TFs. Figure 4B shows five hub genes (CCR7, CXCL10, CCL5, IRF4, and MMP9) targeted to 5 miRNAs (hsa-mir-21-5p, hsa-mir-520g-3p, hsa-mir-125a-5p, and has-mir-15b-5p).

Prediction of candidate drugs

Our analysis revealed 11 potential drugs targeting four hub genes (CXCL10, MMP9, GZMB, and NLRP3) that could be used as common treatments for both diseases. The list of potential drugs is presented in Table II.

Discussion

DM is a condition characterised by long-term, symmetrical muscle weakness and discomfort due to an immune system malfunction (2). It is often accompanied by ILD (8). Unfortunately, the exact mechanisms underlying the association between DM and ILD remain unclear. This study's bioinformatic approach may help identify new therapeutic targets for the treatment of both DM and ILD.

At first, we analysed skin samples from GSE46239 and GSE47162 and found 122 mutual DEGs (67 up-regulated and 55 down-regulated) between DM and ILD. Next, we conducted GO analysis to investigate the potential functional roles of these DEGs. The top GO term for BP was "inflammatory response", while the primary CC were "cytoplasm"

Table II. Core genes and associated drugs.

Gene	Drug
CXCL10	RITONAVIR
CXCL10	ZIDOVUDINE
CXCL10	STAVUDINE
CXCL10	TESTOSTERONE
CXCL10	OXALIPLATIN
CXCL10	ATROPINE
CXCL10	ATORVASTATIN
CXCL10	METHYLPREDNISOLONE
MMP9	BEVACIZUMAB
GZMB	HEXACHLOROPHENE
NLRP3	HEXACHLOROPHENE

and "cytosol". Regarding MF, the top GO terms were "protein binding", and "GTP binding". These results showed that DEGs were associated with inflammatory, which was consistent with the other research (21). Additionally, we utilised KEGG pathway analysis to identify cellular and organismal level functions from the dataset. The top KEGG pathways were "NOD-like receptor signalling pathway" and "TNF signalling pathway". The TNF signalling pathway plays a crucial role in regulating immune responses, including the activation and proliferation of immune cells, cytokine production, and cell death. Dysregulation of TNF signalling can lead to the development of immune system diseases (22). Presently, TNF-α inhibitors that have been given the green light by clinical experts have demonstrated considerable effectiveness in a range of autoimmune disorders, with fresh TNF-α signalling inhibitors now being assessed in a clinical setting (23).

Furthermore, we constructed PPI networks using DEGs, comprising 316 edges and 72 nodes. Using the CytoHubba plugin, we identified the top ten hub genes as CD163, GZMB, IRF4, CCR7, MMP9, AIF1, CXCL10, CCL5, IRF8, and NLRP3.

The glycoprotein CD163 is expressed by monocytes located at the periphery and activated macrophages. Macrophages activating causes the release of a soluble form of it into the peripheral blood during acute or chronic inflammation (24). Studies have revealed that serum concentrations of CD163 are positively associated with DM, particularly in patients who test positive for anti-MDA5, and with ILD related to PM or DM (25).

GZMB has been the subject of the most scientific inquiry among the five human granzymes (A, B, H, K, and M), and it primarily mediates cell apoptosis (26). Previous studies have suggested the role of GZMB in collagen remodelling, scarring, and fibrosis, such as cardiac fibrosis (27). In our study, we identified GZMB as a hub gene and the related drug molecule, hexachlorophene, which is mainly used as a disinfectant.

The homing of distinct types of T cells and antigen-presenting dendritic cells to the lymph nodes is heavily reliant on CCR7 (28). CCR7 protein expression was found in surgical lung biopsies from patients with ILD (29). Similarly, up-regulation of expression of CCR7 on plasmacytoid DCs, both intramuscular and circulating, has been identified in DM patients (29).

The MMPs family, which includes 20 metallopeptidases, including gelatinases, collagenases, and membrane-type MMPs. High concentrations of MMP-9 have been associated with numerous inflammatory, autoimmune, degenerative, and cancerous illnesses (30). Recent studies into MMP-9 have moved away from primarily focusing on cancer to now exploring its effects on vascular and inflammatory diseases. High amounts of MMP-9 have been observed in both DM and ILD, especially in ILD related to connective tissue diseases (31). Therefore, MMP-9 could be involved in the pathogenesis of both DM and ILD, making it a potential biomarker for the use of its inhibitors in treatment.

Allograft inflammatory factor 1 (AIF-1) is an intracellular protein that binds calcium in EF-hand motifs. The HLA class III genomic region was the source of the initial discovery of this molecule, which was identified in macrophages near coronary arteries in a rat heart transplant experiment (32). In animal models of lung fibrosis induced by bleomycin, AIF-1 was found to be present in lung tissue, particularly in macrophages, and it triggered an increase in TGF-β levels, a crucial element in the fibrosis progression. Furthermore, the examination of lung biopsies from those with systemic sclerosis-related ILD revealed the presence of AIF-1 in vessels, macrophages, and T cells (33).

The chemokine CXCL10, which can trigger chemotaxis, foster the differentiation of immune cells, and lead to tissue extravasation, has been recognised as a likely biomarker for both DM and juvenile dermatomyositis due to its correlation with the intensity of the diseases' activity (34). CXCL10 has also been proposed as a novel biomarker for ILD, especially rheumatic disease-related ILD (34). Our study identified CXCL10 as one of the top 10 hub genes in datasets for DM and ILD and enriched with eight candidate drug molecules for therapy.

IRFs are a group of transcription factors that are essential to the immune system, such as the growth and diversification of immune cells, and the management of reactions to pathogens (35). IRF4, and IRF8 are critical in controlling myeloid cell development and characteristics, and therefore have a major impact on inflammatory responses (36). However, the specific interferon regulatory factors (IRFs) involved in DM and ILD and their respective roles in the pathogenesis of both diseases are still unclear.

One of the members of the mammalian chemokine system, CCL5 participates in diverse cancer metastasis and progression (37) and has been shown to mediate the infiltration of inflammatory cells in both ILD and DM (38). However, more large-sampled evidence is still lacking, and further research is needed.

NLRP3, the most well-known inflammasome, is composed of the NLRP3 protein, a member of the NOD-like receptor family (39). Studies have demonstrated the link between NLRP3 inflammasome and the onset and progression of various diseases, including metabolic disorders, and rheumatic diseases such as rheumatoid arthritis, DM/polymyositis (PM), and systemic sclerosis (40). Recent research has also indicated the potential role of NLRP3 inflammasome in the pathogenesis of pulmonary fibrosis, a typical pathological change in ILD (41). Therefore, targeting the NLRP3 inflammasome could be a potential therapeutic option in both DM and ILD, and drugs such as anakinra (42), an IL-1 inhibitor enriched by NLRP3, could be candidate options in the future.

To understand the development of diseases, it is crucial to examine the reciprocal interactions between mutual DEGs and TFs and miRNAs. TFs genes can recognise specific DNA sequences and modulate the transcription and expression of target genes. In our study, we found that 122 TFs and five miRNAs are implicated in regulating hub genes, suggesting a strong correlation between them. These findings highlight the critical role of TFs genes and miRNAs in the regulation of hub genes and the potential impact they may have on disease development.

In this study, we identified 11 promising drugs/molecules for potential therapy, with eight of them being enriched from CXCL10 and the rest from MMP9, GZMB, and NLRP3, respectively. Among these, ritonavir (43), zidovudine (44), and stavudine (45) are antiviral drugs used for combating HIV infection, while Methylprednisolone is a widely used drug for treating ILD and DM, although its prolonged use is associated with certain side-effects (46).

However, this study has several limitations. First, while bioinformatics approaches are powerful for identifying potential biomarkers and therapeutic targets, the findings require experimental validation to confirm the roles of the identified genes and pathways in the pathogenesis of DM and ILD. Although the analysis provided insights into shared molecular mechanisms, it is based on data from publicly available datasets, which may not fully represent the heterogeneity of patients with these diseases. Additionally, the study relies on skin samples, which may not capture all relevant molecular features, particularly those associated with pulmonary involvement in ILD. Further research, including tissue-specific studies and clinical validation, is needed to strengthen these findings.

The innovative aspect of this study lies in the application of bioinformatics to explore the molecular intersection between DM and ILD. By integrating data from multiple sources and using advanced tools like GEO2R, PPI networks, and pathway enrichment analyses, this study provides a comprehensive view of the shared genetic landscape of these diseases. The identification of common DEGs and pathways not only improves our understanding of their pathogenesis but also highlights potential therapeutic targets that could be explored in clinical trials.

Conclusion

We employed bioinformatics methods to investigate the correlation between DM and ILD in our study, which resulted in the discovery of ten hub genes and potential medications that could serve as a promising therapy for DM and ILD in the future.

References

1. FORNARO M, GIROLAMO F, GIANNINI M *et al*.: Clinical, histologic and prognostic features of clinically amyopathic dermatomyositis. *Clin Exp Rheumatol* 2024; 42(2): 288-94. https://doi.org/10.55563/clinexprheumatol/kgpnbq
2. DEWANE ME, WALDMAN R, LU J: Dermatomyositis: Clinical features and pathogenesis. *J Am Acad Dermatol* 2020; 82(2): 267-81. https://doi.org/10.1016/j.jaad.2019.06.1309
3. O'CONNELL KA, LACHANCE AH: Dermatomyositis. *N Engl J Med* 2021; 384(25): 2437. https://doi.org/10.1056/nejmicm2033425
4. CONTICINI E, DOURADO E, BOTTAZZI F *et al*.: Idiopathic inflammatory myopathies: one year in review 2023. *Clin Exp Rheumatol* 2024; 42(2): 213-24. https://doi.org/10.55563/clinexprheumatol/dh5o6c
5. CHU LL, ROHEKAR G: Dermatomyositis. *CMAJ* 2019; 191(12): E340. https://doi.org/10.1503/cmaj.180947
6. WALDMAN R, DEWANE ME, LU J: Dermatomyositis: Diagnosis and treatment. *J Am Acad Dermatol* 2020; 82(2): 283-96. https://doi.org/10.1016/j.jaad.2019.05.105
7. SASAKI H, KOHSAKA H: Current diagnosis and treatment of polymyositis and dermatomyositis. *Mod Rheumatol* 2018; 28(6): 913-21. https://doi.org/10.1080/14397595.2018.1467257
8. LONG K, DANOFF SK: Interstitial lung disease in polymyositis and dermatomyositis. *Clin Chest Med* 2019; 40(3): 561-72. https://doi.org/10.1016/j.ccm.2019.05.004
9. WIJSENBEEK M, SUZUKI A, MAHER TM: Interstitial lung diseases. *Lancet* 2022; 400(10354): 769-86. https://doi.org/10.1016/S0140-6736(22)01052-2
10. PERELAS A, SILVER RM, ARROSSI AV, HIGHLAND KB: Systemic sclerosis-associated interstitial lung disease. *Lancet Respir Med* 2020; 8(3): 304-20. https://doi.org/10.1016/S2213-2600(19)30480-1
11. PODOLANCZUK AJ, WONG AW, SAITO S, LASKY JA, RYERSON CJ, EICKELBERG O: Update in interstitial lung disease 2020. *Am J Respir Crit Care Med* 2021; 203(11): 1343-52. https://doi.org/10.1164/rccm.202103-0559up
12. GEORGE PM, SPAGNOLO P, KREUTER M *et al*.: Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. *Lancet Respir Med* 2020; 8(9): 925-34. https://doi.org/10.1016/S2213-2600(20)30355-6
13. WONG AW, RYERSON CJ, GULER SA: Progression of fibrosing interstitial lung disease. *Respir Res* 2020; 21(1): 32. https://doi.org/10.1186/s12931-020-1296-3
14. ANTONIOU KM, MARGARITOPOULOS GA, TOMASSETTI S, BONELLA F, COSTABEL U, POLETTI V: Interstitial lung disease. *Eur Respir Rev* 2014; 23(131): 40-54. https://doi.org/10.1183/09059180.00009113
15. DALMER TRA, CLUGSTON RD: Gene ontology enrichment analysis of congenital diaphragmatic hernia-associated genes. *Pediatr Res* 2019; 85(1): 13-9. https://doi.org/10.1038/s41390-018-0192-8
16. KANEHISA M, FURUMICHI M, TANABE M, SATO Y, MORISHIMA K: KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017; 45(D1): D353-D61. https://doi.org/10.1093/nar/gkw1092
17. DENNIS G, SHERMAN BT, HOSACK DA *et al*.: DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol* 2003; 4(5): P3.
18. CHIN C-H, CHEN S-H, WU H-H, HO C-W, KO M-T, LIN C-Y: cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol* 2014; 8 Suppl 4(Suppl 4): S11. https://doi.org/10.1186/1752-0509-8-S4-S11
19. CASTRO-MONDRAGON JA, RIUDAVETS-PUIG R, RAULUSEVICIUTE I *et al*.: JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Res* 2022; 50(D1): D165-D73. https://doi.org/10.1093/nar/gkab1113
20. WAGNER AH, COFFMAN AC, AINSCOUGH BJ *et al*.: DGIdb 2.0: mining clinically relevant drug-gene interactions. *Nucleic Acids Res* 2016; 44(D1): D1036-D44. https://doi.org/10.1093/nar/gkv1165
21. CONKLIN LS, MERKEL PA, PACHMAN LM *et al*.: Serum biomarkers of glucocorticoid response and safety in anti-neutrophil cytoplasmic antibody-associated vasculitis and juvenile dermatomyositis. *Steroids* 2018; 140: 159-66. https://doi.org/10.1016/j.steroids.2018.10.008
22. KALLIOLIAS GD, IVASHKIV LB: TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* 2016; 12(1): 49-62. https://doi.org/10.1038/nrrheum.2015.169
23. JANG D-I, LEE AH, SHIN H-Y *et al*.: The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *Int J Mol Sci* 2021; 22(5). https://doi.org/10.3390/ijms22052719
24. NIELSEN MC, HVIDBJERG GANTZEL R, CLÀRIA J, TREBICKA J, MØLLER HJ, GRØNBÆK H: Macrophage activation markers, CD163 and CD206, in acute-on-chronic liver failure. *Cells* 2020; 9(5). https://doi.org/10.3390/cells9051175
25. LU X, PENG Q, WANG G: Biomarkers of disease activity in dermatomyositis. *Curr Opin Rheumatol* 2022; 34(6): 289-94. https://doi.org/10.1097/bor.0000000000000905

26. TURNER CT, LIM D, GRANVILLE DJ: Granzyme B in skin inflammation and disease. *Matrix Biol* 2019; 75-76: 126-40. https://doi.org/10.1016/j.matbio.2017.12.005
27. SHEN Y, CHENG F, SHARMA M *et al*.: Granzyme B deficiency protects against angiotensin II-induced cardiac fibrosis. *Am J Pathol* 2016; 186(1). https://doi.org/10.1016/j.ajpath.2015.09.010
28. SALEM A, ALOTAIBI M, MROUEH R, BASHEER HA, AFARINKIA K: CCR7 as a therapeutic target in cancer. *Biochim Biophys Acta Rev Cancer* 2021; 1875(1): 188499. https://doi.org/10.1016/j.bbcan.2020.188499
29. BRANDUM EP, JØRGENSEN AS, ROSENKILDE MM, HJORTØ GM: Dendritic cells and CCR7 expression: an important factor for autoimmune diseases, chronic inflammation, and cancer. *Int J Mol Sci* 2021; 22(15): 8340. https://doi.org/10.3390/ijms22158340
30. MONDAL S, ADHIKARI N, BANERJEE S, AMIN SA, JHA T: Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. *Eur J Med Chem* 2020; 194: 112260. https://doi.org/10.1016/j.ejmech.2020.112260
31. BASSIOUNI W, ALI MAM, SCHULZ R: Multifunctional intracellular matrix metalloproteinases: implications in disease. *FEBS J* 2021; 288(24): 7162-82. https://doi.org/10.1111/febs.15701
32. BARANZINI N, MONTI L, VANOTTI M *et al*.: AIF-1 and RNASET2 play complementary roles in the innate immune response of medicinal leech. *J Innate Immun* 2019; 11(2): 150-67. https://doi.org/10.1159/000493804
33. ZHAO Y-Y, YAN D-J, CHEN Z-W: Role of AIF-1 in the regulation of inflammatory activation and diverse disease processes. *Cell Immunol* 2013; 284(1-2): 75-83. https://doi.org/10.1016/j.cellimm.2013.07.008
34. TOKUNAGA R, ZHANG W, NASEEM M *et al*.: CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy. *Cancer Treat Rev* 2018; 63: 40-47. https://doi.org/10.1016/j.ctrv.2017.11.007
35. COLLIN M, BIGLEY V: Human dendritic cell subsets: an update. *Immunology* 2018; 154(1). https://doi.org/10.1111/imm.12888
36. JEFFERIES CA: Regulating IRFs in IFN driven disease. *Front Immunol* 2019; 10: 325. https://doi.org/10.3389/fimmu.2019.00325
37. MARQUES RE, GUABIRABA R, RUSSO RC, TEIXEIRA MM: Targeting CCL5 in inflammation. *Expert Opin Ther Targets* 2013; 17(12): 1439-60. https://doi.org/10.1517/14728222.2013.837886
38. ZENG Z, LAN T, WEI Y, WEI X: CCL5/CCR5 axis in human diseases and related treatments. *Genes Dis* 2022; 9(1): 12-27. https://doi.org/10.1016/j.gendis.2021.08.004
39. KELLEY N, JELTEMA D, DUAN Y, HE Y: The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci* 2019; 20(13). https://doi.org/10.3390/ijms20133328
40. COLL RC, SCHRODER K, PELEGRÍN P: NLRP3 and pyroptosis blockers for treating inflammatory diseases. *Trends Pharmacol Sci* 2022; 43(8): 653-68. https://doi.org/10.1016/j.tips.2022.04.003
41. MANGAN MSJ, OLHAVA EJ, ROUSH WR, SEIDEL HM, GLICK GD, LATZ E: Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat Rev Drug Discov* 2018; 17(8): 588-606. https://doi.org/10.1038/nrd.2018.97
42. TEGTMEYER K, ATASSI G, ZHAO J, MALONEY NJ, LIO PA: Off-Label studies on anakinra in dermatology: a review. *J Dermatolog Treat* 2022; 33(1): 73-86. https://doi.org/10.1080/09546634.2020.1755417
43. LAMB YN: Nirmatrelvir plus ritonavir: first approval. *Drugs* 2022; 82(5): 585-91. https://doi.org/10.1007/s40265-022-01692-5
44. BIANCO MDCAD, INACIO LEITE D, SILVA CASTELO BRANCO F *et al*.: The use of zidovudine pharmacophore in multi-target-directed ligands for AIDS therapy. *Molecules* 2022; 27(23): 8502. https://doi.org/10.3390/molecules27238502
45. MAGULA N, DEDICOAT M: Low dose versus high dose stavudine for treating people with HIV infection. *Cochrane Database Syst Rev* 2015; 1(1): CD007497. https://doi.org/10.1002/14651858.cd007497.pub2
46. LUGER TA: Balancing efficacy and safety in the management of atopic dermatitis: the role of methylprednisolone aceponate. *J Eur Acad Dermatol Venereol* 2011; 25(3): 251-8. https://doi.org/10.1111/j.1468-3083.2010.03789.x