Investigation of molecular mechanisms using integrated analysis of transcriptomes and cytokinome in dermatomyositis

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Abstract
Background Pathomechanism of dermatomyositis (DM) remains yet fully elucidated. While several cytokines have been proved to participate in the progress of DM, few studies provided a comprehensive analysis of cytokinome in different DM clinical-serological subgroups and correlation with disease activity as well as interaction with DM tissue lesions.

Methods Transcriptome datasets of DM skin and muscle were obtained from public database. Hub genes and signaling pathways were filtered by bioinformatic software. Serum cytokinome was measured in DM patients with different clinical-serological subgroups and correlation with disease activity indicators was analyzed. Cytokine interaction network was constructed.

Results 6 hub genes, including STAT1, MX1, ISG15, IFIT3, GBP1 and OAS2 were identified as IFN signature in DM. Differently expressed genes (DEGs) identified in the skin and muscle datasets were significantly enriched in the type I interferon signaling pathway, defense response to virus and chemotaxis. 11 cytokines were significantly elevated in patients positive for melanoma differentiation-associated protein (MDA5) antibody. IFN-α, IFN-γ, MIP-1α, IP-10, MCP1, GRO-α, IL-6, IL-18 and IL-1RA were correlated with disease activity. MCP1/MIP-1α/RANTES/MCP2/CCR1 axes were filtered from cytokine interaction network.

Conclusions The complexity of DM immunopathogenesis is mediated through interactions of multiple cytokines and signaling pathways. Type I interferon is the core participant in DM tissue damage. Serum upregulation of IFN-α, IFN-γ, MIP-1α, IP-10, MCP1, GRO-α, IL-6, IL-18 and IL-1RA could be used for disease activity assessment in DM patients positive for MDA5 antibody. Finally, MCP1/MIP-1α/RANTES/MCP2/CCR1 axes mediated monocytes attraction might be novel therapeutic targets in DM by chemokine network analysis.

Background
Dermatomyositis (DM) is a systemic autoimmune disease with multiple organs involvement. Specific features include typical rash and evolving muscle weakness of proximal extremities. Based on myositis specific autoantibodies (MSAs), DM was classified into different clinical-serological subgroups. Clinical manifestations are associated with MSAs[1]. Patients with anti-melanoma
differentiation-associated protein 5 (MDA5) antibody frequently have typical rash, including heliotrope rash, Gottron sign and Gottron papules, and have high incidence of interstitial lung disease (ILD)[2, 3]. In an eastern Asian myositis cohort, 74% patients positive for MDA5 antibody have rapidly progressive ILD[4]. Patients with transcription intermediary factor 1γ antibody (TIF1γ) have high risk of cancer apart from classic cutaneous lesions (“red on white”) but less likely have ILD[5, 6].

Immunopathogenesis in DM is quite complicated [7, 8]. Numerous studies unveiled that type I interferon (IFN) and several other cytokines were significantly correlated with disease activity in DM[9, 10]. IP-10/CXCR3 axis was found co-upregulated in muscle samples[11]. However, few studies profiled a broad spectrum cytokinome in different DM clinical-serological subgroups. In addition, investigation of cytokine interactome associated with DM tissue lesion remained lacking.

Microarray technology has been widely applied in biomedical researches. How to extract and reorganize valuable information comprehensively and accurately from microarray data and obtain disease-focused genes and signaling pathways have become the main challenge in microarray clinical application. Integrated data from multi-omics experiments may contribute to coping with this challenge and promoting our understanding of complex diseases.

We identified bub genes and signaling pathways by analyzing DM skin and muscle transcriptomes. Cytokines and chemokines mediated signaling pathways were significantly activated. Afterwards, we found similar pattern by cytokinome analysis. Disease activity associated cytokines in MDA5 group were determined. Finally, we filtered principal chemokine axes and interaction pairs from chemokine interaction network.

Methods

Collection of GEO datasets and data processing of DM

Three gene expression profiles were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) DataSets: GSE46239[12], GSE1551[13] and GSE39454[14]. GSE46239 dataset was based on the GPL570(Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix, Inc, CA, Santa Clara) platform and included 48 dermatomyositis skin samples and 4 normal skin samples. GSE1551 dataset was based on the GPL96(Affymetrix Human Genome
U133A Array, Affymetrix, Inc, CA, Santa Clara) platform and included 13 dermatomyositis muscle samples and 10 normal muscle samples. GSE39454 dataset was based on GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix, Inc, CA, Santa Clara) platform and included 8 dermatomyositis muscle samples, 10 inclusion body myositis, 8 polymyositis and 5 immune mediated necrotizing myopathy muscle samples. Our study selected 48 skin samples, 21 muscle samples and total 16 parallel normal samples for the bioinformatics analysis (Three normal controls were excluded for aberrant expression value in dataset 1551).

Perl 5.0 was applied to convert the expression profiles from probes into gene symbols. We performed normalization by imputing the missing values and averaging the expression values of multiple probes corresponded with the same gene. Limma package in R software was used to filter DEGs between dermatomyositis and normal samples in each dataset. P value<0.05 and | log FC |>1(fold change) were considered statistically significant. Heml software was applied to construct DEG heatmaps. Venn diagram was used to find the overlapped DEGs between the two muscle datasets, GSE1551 and GSE39454.

**GO and KEGG pathway enrichment analysis**

We performed the gene ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analysis on DAVID to translate DEG lists into the functional profiles. Biological process (BP), molecular function (MF) and cellular component (CC) were detected. Terms with adjusted P value <0.05 and gene counts>2 were considered statistically significant.

**PPI network construction and hub genes identification**

Protein-protein interaction (PPI) networks were described on String database. Interaction score>0.4 was set. Cytoscape software was used to display PPI networks. Top ten DEGs with high degree value were filtered out as hub genes.

**Patients**

Serum samples of 40 DM patients enrolled into the myositis cohort study in Peking Union Medical College Hospital from 2017 to 2019 were collected and stored at -80 degrees. DM patients fulfilled the Bohan and Peter criteria [15]. DM patients were classified into active or stable group using the
following criteria. Patients who were prescribed with high doses daily corticosteroids (more than 40mg/day) alone and in combination with high doses of other immunosuppressives or intravenous gamma globulin (IVIG) or had one of the following items are thoughted to be active: i. acute cutaneous lesions (e.g. erythroderma or erythematous rashes with erosive changes) ii. severe muscle inflammation (serious muscle weakness and high serum CK) iii. progressive interstitial lung disease (dyspnea, cough or dyspnea on exertion due to ILD, parenchymal abnormalities on HRCT and pulmonary function test showed FVC or DLCO <70%). Patients whose clinical signs and symptoms had largely disappeared and corticosteroids had started to reduce or had reduced to less than 15mg/day were considered at clinical stabilization. Disease activity score was retrospectively applied according to the medical records. The Peking Union Medical College Hospital Review Board approved this study and informed consent was obtained from each patient.

**Cytokines profiling**

Serum levels of 34 cytokines including MIP-1α, SDF-1α, IL-27, IL-1β, IL-2, IL-4, IL-5, IP-10, IL-6, IL-7, IL-8, IL-10, Eotaxin, IL-12p70, IL-13, IL-1RA, RANTES, IFN-gamma, GM-CSF, TNF-α, MIP-1β, IFN-α, MCP-1, IL-9, TNF-β, GRO-α, IL-1α, IL-23, IL-15, IL-18, IL-21, IL-22, IL-31 and IL-17α were determined by KingMed diagnostics (Beijing, China) using ProcartaPlex Human Cytokine & Chemokines Panel, Affymetrix (eBioscience, EPX340-12167-901).

**Statistical analysis**

IBM SPSS version 25 and GraphPad Prism 8 were applied for the cytokine analyses. For continuous variables, Shapiro-Wilktest was used to test the normality. Non-normal data were described by median (quartile) and compared by Kruskal-Wallis. Bonferroni correction was applied to correct for multiple comparisons. P value less than 0.05 was considered statistically significant. Principal component analysis (PCA) was performed on the ln-transformed data using Clustvis[16]. Pearson’s correlation was conducted for some data.

**Results**

**Identification of DEGs related to skin and muscle damage in DM**

224 DEGs, including 180 upregulated and 44 downregulated genes in skin dataset GSE46239 were
identified. We screened 472 and 177 DEGs respectively in muscle dataset GSE1551 and GSE39454. Afterwards we filtered 101 overlapped DEGs in the two muscle datasets (Additional file 1: Table 1). Heatmaps of partial DEGs were made (Fig. 1). Gene symbols of DEGs with the log FC and P value in each dataset were attached (Additional file 1: Table2-4).

**GO and KEGG pathway enrichment analysis of DEGs in the skin dataset**

We analyzed the Gene ontology and KEGG pathway to elucidate the biological meaning of DEGs. In regard of skin involvement, top 11 terms enriched in BP included type I interferon signaling pathway, defense response to virus, response to virus, negative regulation of viral genome replication, immune response, interferon-γ mediated signaling pathway, inflammatory response, response to interferon-beta and alpha, innate immune response and chemotaxis (Additional file 2: Figure S1a.). Major enrichment in CC is composed of extracellular space, extracellular region, blood microparticle and external side of plasma membrane. Primary enrichment in MF consists of double-stranded RNA binding, 2′-5′-oligoadenylate synthetase activity, heparin binding and chemokine activity (Additional file 2: Figure S1b). KEGG pathway analysis showed that the DEGs were enriched in the Influenza A, Measles, Complement and coagulation cascades, Pertussis, Staphylococcus aureus infection, Herpes simplex infection, Hepatitis C, Toll-like receptor signaling pathway, Cytosolic DNA-sensing pathway, Chagas disease (American trypanosomiasis), Chemokine signaling pathway, Cytokine-cytokine receptor interaction and NF-kappa β signaling pathway (Additional file 2: Figure S1c).

**GO and KEGG pathway enrichment analysis of DEGs in the muscle datasets**

The analytical results of the overlapped DEGs in muscle datasets were similar with those in skin dataset. Top 15 BP included type I Interferon signaling pathway, defense response to virus, response to virus, Interferon-Gamma-mediated signaling pathway, negative regulation of viral genome replication, Immune response, antigen processing and presentation of exogenous peptide antigen via MHC Class I tap-independent, response to Interferon-beta, innate Immune response, antigen processing and presentation of peptide antigen via MHC Class I, antigen processing and presentation of exogenous peptide antigen via MHC Class I tap-dependent, defense response, chemotaxis, inflammatory response and response to interferon-alpha (Fig.2a). CC analysis revealed that DEGs
were enriched in the MHC Class I protein complex, integral component of luminal side of endoplasmic reticulum membrane, extracellular region, ER to Golgi transport vesicle membrane, phagocytic vesicle membrane, cytosol and early endosome membrane. Primary enrichment in MF comprised of peptide antigen binding, double-stranded RNA binding, TAP binding, chemokine activity, 2'-5'-oligoadenylate synthetase activity, CXCR3 chemokine receptor binding, single-stranded RNA binding and heparin binding (Fig.2b). KEGG pathway enrichment analysis revealed that DEGs were enriched in herpes simplex infection, influenza A, measles, phagosome, graft-versus-host disease, allograft rejection, antigen processing and presentation and Epstein-Barr virus infection (Fig.2c).

**PPI network analysis and hub genes identification**

There were 208 nodes and 1901 edges in skin-associated PPI network (Additional file 2: Figure S2) and the top 10 hub genes based on interaction degree value were screened out, including STAT1, CXCL10, IRF7, MX1, IFIH1, ISG15, IFIT3, IFIT1, GBP1 and OAS2. Muscle-associated PPI network consisted of 98 nodes and 1008 edges (Fig.3). Hub genes included STAT1, MX1, GBP1, IFIT3, ISG15, OAS2, OAS1, OAS3, RSAD2 and IFIT2. Overlapped hub genes, including STAT1, MX1, ISG15, IFIT3, GBP1 and OAS2 were filtered.

**Cytokines profiling in DM**

Considering the results of bioinformatics analysis, some cytokines, chemokines and related receptors (e.g. CCR1, CCR7, CCL10 and CCL2) were elevated in DM skin and muscle tissues, we profiled serum cytokines in DM patients by using cytokines and chemokines panel to find difference in clinical-serological subgroups and disease activity related cytokines as well as construct cytokine interaction network.

40 DM were enrolled in this study. Clinical features of the patients were shown (Table 1). The median age was 44. 20 patients with anti MDA5 antibody were divided into the active and stable group according to the above criteria. 10 patients positive for anti TIF1γ antibody, 10 patients negative for any myositis specific antibody and 10 healthy controls (HCs) were also included. The concentration of 34 cytokines were compared in different groups (Table 2). One sample was excluded for abnormal value.
PCA was used to describe the difference of cytokine levels in DM subgroups generally (Fig.4a). The first principal component separated MDA5 group and HCs. Separation was also observed between the active and stable group in MDA5 DM patients. IFN-α, IFN-γ, IL-18, IL-6, MCP1, MIP-1α, RANTES, GRO-α, IL-8, IP-10 and IL-1RA were significantly higher in active group of patients with MDA5 antibody than HCs (Fig.4). In contrast with active group, IFN-α, IFN-γ, MCP1, IP-10 and IL-1RA were found significantly lower in MDA5-antibody stable group. Higher level of IP-10 was also found in patients with TIF1γ antibody than HCs. However, IFN-α and IFN-γ were not significantly higher in TIF1γ antibody group (Fig.4b-c).

**Correlation analysis and chemokine network construction**

Significantly raised cytokines in MDA5 active group were classified into four groups, activation of Th1 type response and Th2 type response, chemokines and anti-inflammation cytokine (Fig.5a).

Correlation analysis between cytokine levels and clinical activity indicators in DM positive for MDA5 antibody showed IFNA, IFNG, IL18, CCL2(MCP1), CCL3(MIP-1α), CXCL1(GRO-α), CXCL10(IP-10) and IL1RA were significantly correlated with disease activity score in MDA5 active group (Fig.5b).

Finally, we constructed chemokine interaction network in DM patients by importing DEGs associated with chemotaxis and upregulated cytokines into String database. IFNG and IL18 as well as CXCL9, CCR1 and CCR7 with top degree value in serum and tissues were selected respectively as the hub cytokines. CXCL10, CCL2 and CCL5 were co-upregulated in DM tissues and serum. Interactions based on lab experimental evidence were screened and marked, including CXCL8-CCL2, CCL8-CXCL8, CCL8-CR1, CCR1-CCL2, CCL5-CCR1, CCL5-CXCL8, CCL5-ACKR4, CCL3-CCR1, CCL8-ACKR4, CCL8-CCL2, ACKR4-CCL2, CCL5-CCL2, CCL5-CXCL10, CCL5-CXCL9, CXCL11-CXCL10, CXCL9-CXCL10, CXCL9-CXCL11, CCL8-CXCL11, CXCL9-CCL2, CCL8-CXCL10, CCL5-CXCL11 and CCL5-CXCL1(Fig.6).

CCL8/CCL2/CCL5/CCL3/CCR1 and CCL5/CCL8/CCL2/ACKR4 axes were determined.

**Discussion**

In this study, we found 6 hub genes and signaling pathways in DM by analyzing microarray datasets and detected disease activity related cytokines in MDA5 group by cytokines profiling and constructed the cytokine interaction network. According to the analytical outcomes of skin and muscle datasets,
the DEGs significantly enriched in the type I interferon signaling pathways, anti-virus response and chemotaxis. In addition, we filtered six hub genes with high interaction degree both in the skin and muscle datasets, including STAT1, MX1, ISG15, IFIT3, GBP1 and OAS2.

STAT1 (signal transducer and transcription activator) is an important transcription factor participating in the interferon signaling pathway. Upon interferon \( \alpha \) binding to IFNAR, the receptors dimerization results in juxtaposition and activation of JAK1 and TYK2. Afterwards, STAT1 and STAT2 are recruited and phosphorylated [17]. Activated STAT1 and STAT2 associate with IRF-9 to form a complex and translocate into the nucleus to activate the transcription of IFN-stimulated genes (ISG), including MX1, ISG15, IFIT3, GBP1 and OAS2 for antivirus response [18, 19].

Recent studies provided the evidence that patients with heterozygous STAT1 gain-of-function mutations are inclined to have autoimmune diseases, including hypothyroidism, type 1 diabetes, blood cytopenia, systemic lupus erythematosus, and Takayasu arteritis [20, 21]. According to the basic study, STAT1 may be required for the formation of spontaneously developed germinal centers in which autoreactive B cells proliferate and generate pathogenic autoantibody to initiate autoimmunity [22].

The six hub genes which we identified are associated with the interferon signature and partly consistent with the previous studies[23, 24]. Interferon plays an important role in the pathogenesis of DM and is correlated with disease severity. There was evidence proved that ROS production and mitochondrial malfunctions induced by the interferon beta may contribute to the muscle inflammation[25]. In vitro, JAK inhibitor prevented the IFN-\( \beta \) induced myotube atrophy and endothelial cells damage by blocking the type I interferon signal pathway [26]. Oral JAK inhibitor, Tofacitinib, improved the recalcitrant cutaneous manifestation and pulmonary involvement in DM patients [27–29].

Interferon score first mentioned in systemic lupus erythematosus is growing to be a promising tool for DM investigation. Although interferon stimulated genes expressed variously in different tissue, common genes were always upregulated. The six hub genes which we identified might constitute the exclusive interferon signature for DM study.
In addition, we also payed our attention to the downregulated DEGs filtered from the muscle datasets. RHOBTB1 is one of family members of the Rho small GTPases. Several studies found RHOBTB1 might participate in suppressing cancer cell invasion. [30, 31]. Interestingly, it has been reported that RHOBTB1 protects vascular smooth muscle dysfunction and promotes cardiomyocyte proliferation [32, 33]. Downregulated RHOBTB1 in skeleton muscle samples of DM patients might be associated with the pathogenesis of muscle atrophy and vascular inflammation. In addition, TFRC, encoding the transferrin receptor, highly expressed in the fast proliferating cells for iron uptake and regulated by complex mechanisms, was downregulated in DM muscle tissue, indicating that iron homeostasis was broken. Iron metabolism disorder might be the cause or outcome of muscle damage.

Given that chemotaxis pathway was significantly enriched by DEGs apart from interferon signaling pathway, antivirus response and antigen presentation, we profiled the cytokines and chemokines in DM patients and found multiple cytokines were upregulated in MDA5 group and correlated with disease activity score.

In MDA5 active group, upregulated serum IFN α, IFN γ and IFN γ production inducer IL18 facilitated the Th1 type response, activating the cell mediated immunity against microbial infection. However, IFN overexpression contributes to the pathogenesis development in DM as well. Moreover, increased serum IL18 was correlated with serum aberrant CK reflecting muscle dysfunction (r = 0.58, p = 0.0116) in MDA5 active group. Several studies previously demonstrated that IL18 highly expressed in muscle and serum of DM patients[34–37] but few evidence regarding the IL8 level in different serum-subgroups in DM. In our study, elevated serum IL18 was observed only in the MDA5 active group and correlated with DAS significantly (r = 0.69, p = 0.001).

Prototypical Th2 type response associated cytokines, including IL4, IL5, IL10 and IL13 were not significantly upregulated in DM patients, suggesting that DM might also be dominated by the Th1-tilted response like many other autoimmune diseases [38]. IL6, a pleiotropic cytokine with various functions, was highly expressed in MDA5 active group as well. Sean Diehl reported that IL6 inhibits Th1 differentiation and IFN γ expression by upregulating suppressor of cytokine signaling (SOCS1) expression in activated T cells and plays an important role in balancing Th1/Th2 response[39].
CCL2, CCL3 and CCL5 attracts and activates monocytes into the inflammation environment and aggravates the tissue destruction. In addition, CCL2 and CCL5 were proved to act synergistically to attract monocytes and/or T cells [40]. We detected CCL2, CCL3 and CCL5 were promoted in serum of active DM patients with MDA5 antibody and receptor CCR1 was elevated in DM skin and muscle tissues, indicating that CCL3/CCL5/CCR1 signaling pathway mediated monocytes attraction might play an important role in DM skin and muscle damage. CCL2 was significantly correlated with the Disease Activity Score in MDA5 active group and might be a potential biomarker for disease activity assessment \((r = 0.7513, p = 0.0002)\).

CXCL1/CXCL8 are neutrophil attractant and play an important role in lung injury[41, 42]. Elevation of CXCL1/CXCL8 in MDA5 active group might implicate the pathogenesis in interstitial lung disease of MDA5 active patients. IP-10 induced by IFN affects the development of skin lesion in DM by recruiting CXCR3 autoreactive T cells [43]. Blocking IFN chemokines might be the novel therapeutic strategy for DM.

Apart from the pro-inflammatory cytokines, we detected that IL-1RA was also highly expressed in MDA5 active patients with immunosuppressive effect by blocking the IL-1 signaling pathway. IL-1RA produced by hepatocytes is regulated by proinflammatory cytokines as an acute-phase protein[44]. Upregulation of IL-1RA in MDA5 active group might implicate the acute inflammation modulation and damage recovery.

Finally, we found MCP1/MIP-1α/RANTES/MCP2/CCR1 and RANTES/MCP2/MCP1/ACKR4 axes were highly expressed from chemokine interaction network analysis. The former axes mediated monocytes arrest might contribute to tissue destruction, the latter, on the contrary, could regulate chemokine level and location by ligand internalization. Chemokine interaction can escalate or block downstream signaling pathways. However, biological meanings of most chemokine interaction pairs identified in DM are unspecified and need to be investigated.

In our study, we found the IFN signature in DM patients by bioinformatics analysis and discovered the disease activity associated cytokines in MDA5 group by cytokines profiling. In addition, we constructed chemokine network in DM patients by combining the results comprehensively which
might shed light on the further pathogenesis study in DM. We have several limitations in our study. First, DEGs filtered from the datasets need to be validated. Secondly, serum was stored in -80°C until used and some cytokines may become undetectable. Thirdly, DAS was retrospectively assessed. Finally, the number of patients in different DM serum subgroups is small and difference were not observed significantly except for MDA5 group.

Conclusions
In summary, we identified core genes and signaling pathways in DM by transcriptome analysis. Cytokinome profiling indicated that IFN-α, IFN-γ, IL-18, MIP-1α, IP-10, MCP1, GRO-α and IL-1RA might be potential biomarkers for disease activity assessment in MDA5 group. MCP1/MIP-1α/RANTES/MCP2/CCR1 axes mediated monocytes attraction might play an important role in the pathogenesis in DM skin and muscle destruction. These findings could assist in clinical diagnosis and become potential treatment targets for DM patients.

Abbreviations
ALT, Alanine transaminase; AST, Aspartate transaminase; BP, Biological process; CC, cellular component; CRP, C-reactive protein; CK, Creatine Kinase; DM, dermatomyositis; DEGs, Differently expressed genes; DAS, disease activity score; DLCO, Diffusing capacity for carbon monoxide; ESR, Erythrocyte Sedimentation Rate; FVC, forced vital capacity; HCs, healthy controls; ILD, interstitial lung disease; IFN, interferon; GEO, Information Gene Expression Omnibus; GO, gene ontology; KEGG, Kyoto Encyclopedia of Gene and Genome; LDH, Lactate dehydrogenase; MDA5, melanoma differentiation-associated protein; MF, molecular function; MSAs, myositis specific autoantibodies; NCBI, National Center for Biotechnology; PCA, Principal component analysis; PPI, Protein-protein interaction; SF, Serum Ferritin; TIF1γ, transcription intermediary factor; α, Alpha; β, Beta; γ, Gamma

Declarations

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Authors’ contribution
Jingjing Bai designed experiments and wrote the manuscript; Chanyuan Wu assisted with patient
enrollment; Danli Zhong made contributions to the acquisition and analysis of data; Dong Xu revised the manuscript; Qian Wang and Xiaofeng Zeng were responsible for patient enrollment. Qian Wang and Xiaofeng Zeng contributed equally.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

This study was approved by the Peking Union Medical College Hospital Review Board according to the principles of the Declaration of Helsinki, and informed consent was obtained from each patient.

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**Tables**

**TABLE 1** Clinical features of the enrolled patients

| Feature                      | Active(n=10) | Stable(n=9) | Stable(n=10) | Stable(n=10) |
|------------------------------|--------------|-------------|--------------|--------------|
| Age, median (IQR), years     | 44(14)       | 30(75)      | 40            |              |
| Female, n (%)                |              |             |              |              |
| DM, n                        | 40           | 20(50)      |              |              |
| ILD, n (%)                   |              |             |              |              |
| Cancer, n (%)                |              |             |              | 2(5)         |
| Myositis specific antibodies |              |             |              |              |
| Anti-MDA5                    | 20(50)       |              |              |              |
| Anti-TIF1γ                   | 10(25)       |              |              |              |
| Non-MSA                      | 10(25)       |              |              |              |

Abbreviations: IQR, interquartile range; DM, dermatomyositis; ILD, interstitial lung disease; MDA5, melanoma differentiation-associated gene 5; TIF1γ, transcription intermediary factor 1γ; MSA, myositis specific antibodies.

**TABLE 2** Cytokine profiles in DM

| Cytokine   | DM-aMDA5 Active(n=10) Median(IQR) | DM-aMDA5 Stable(n=9) Median(IQR) | DM-aTIF1γ(n=10) Median(IQR) | DM-non MSA Stable(n=10) Median(IQR) |
|------------|-----------------------------------|----------------------------------|----------------------------|----------------------------------|
| IL-2       | 2.60(1.33-5.30)                   | 1.40(1.40-8.25)                  | 1.40(1.40-4.58)              | 1.40(1.33-1.40)                  |
| IL-12p70   | 0.60(0.60-0.60)                   | 0.60(0.60-0.60)                  | 0.60(0.60-0.60)              | 0.60(0.60-0.60)                  |
| IFN-α a    | 0.95(0.40-11.75)                  | 0.1(0.1-0.1)                     | 0.1(0.1-0.1)                 | 0.1(0.1-0.1)                     |
|            | 26.50(14.33-35.53)                | 4.80(4.80-4.80)                  | 4.80(4.80-8.35)              | 4.80(4.80-18.03)                 |

Activation of Th1 type response.
### Activation of Th2 type response

| Cytokine | IL-4 | IL-5 | IL-6 | IL-9 | IL-13 | IL-22 | IL-31 | GM-CSF |
|----------|------|------|------|------|-------|-------|-------|--------|
| IFN-γ   | 20.10(8.51-30.73) | 4.65(4.65-5.55) | 4.65(4.65-4.65) | 4.65(4.65-11.70) | 20.10(8.51-30.73) | 4.65(4.65-5.55) | 4.65(4.65-5.55) | 4.65(4.65-5.55) |
| IL-18   | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) | 1.50(1.50-1.50) |
| TNFα    | 2.65(2.65-5.60) | 2.65(2.65-7.58) | 2.65(2.65-4.76) | 2.65(2.65-2.65) | 2.65(2.65-5.60) | 2.65(2.65-7.58) | 2.65(2.65-4.76) | 2.65(2.65-2.65) |
| TNFβ    | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) | 2.25(2.25-2.25) |

### Activation of Th17 type response

| Cytokine | IL-17 | IL-23 | IL-10 | IL-18 |
|----------|-------|-------|-------|-------|
| IFN-γ   | 1.10(1.10-1.10) | 1.10(1.10-1.10) | 1.10(1.10-1.10) | 1.10(1.10-1.10) |
| IL-6     | 0.90(0.90-0.90) | 0.90(0.90-0.90) | 0.90(0.90-0.90) | 0.90(0.90-0.90) |
| IL-9     | 13.10(1.30-30.4) | 1.30(1.30-9.15) | 1.30(1.30-67.56) | 1.30(1.30-1.30) |
| IL-13    | 0.35(0.35-0.35) | 0.35(0.35-0.35) | 0.35(0.35-0.35) | 0.35(0.35-0.35) |
| IL-22    | 4.90(4.90-32.03) | 4.90(4.90-43.35) | 4.90(4.90-4.90) | 4.90(4.90-40.45) |
| IL-31    | 1.00(1.00-1.00) | 1.00(1.00-1.00) | 1.00(1.00-1.00) | 1.00(1.00-1.00) |
| GM-CSF   | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) |

### Chemokines

| Cytokine | SDF-1α | MCP-1α | IP-10α | IL-8α |
|----------|--------|--------|--------|-------|
| IFN-γ   | 589.50(485.67-726.27) | 207.30(116.18-355.45) | 289.90(178.38-585.57) | 29.75(4.80-156.10) |
| IL-6     | 514.20(318.75-632.80) | 63.70(36.75-126.65) | 47.50(29.85-59.20) | 0.55(0.55-24.15) |
| IL-9     | 390.25(334.52-521.50) | 79.15(51.75-110.55) | 56.20(29.20-255.85) | 2.27(0.55-16.43) |
| IL-13    | 420.55(351.30-858.53) | 50.1(35.08-149.48) | 35.70(16.40-56.68) | 1.75(0.55-12.2) |
| IL-22    | 1.00(1.00-1.00) | 341.20(298.38-485.57) | 420.55(351.30-858.53) | 35.70(16.40-56.68) |
| IL-31    | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) |
| GM-CSF   | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) | 1.75(1.75-1.75) |

### Proliferation and differentiation of immunocytes

| Cytokine | IL-7 | IL-15 | IL-21 |
|----------|------|------|------|
| IFN-γ   | 3.00(1.50-3.33) | 0.70(0.70-2.45) | 0.90(0.90-7.33) |
| IL-6     | 2.10(0.63-4.80) | 0.70(0.70-0.70) | 0.90(0.90-4.05) |
| IL-9     | 1.40(1.08-2.20) | 0.70(0.70-0.70) | 0.90(0.90-9.01) |
| IL-13    | 1.60(0.95-2.58) | 0.70(0.70-0.70) | 0.90(0.89-4.03) |
| IL-22    | 2.20(1.55-5.6) | 0.90(0.90-4.05) | 0.90(0.89-4.03) |

### Anti-inflammatory cytokines

| Cytokine | IL-1RAα | IL-10 |
|----------|--------|------|
| IFN-γ   | 1205.45(511.85-1784.75) | 12.55(10.70-99.85) |
| IL-6     | 240.95(10.70-1001.60) | 41.83(10.7-550.93) |
| IL-9     | 50.00(50.00-50.00) | 0.50(0.50-0.50) |
| IL-22    | 0.50(0.50-0.625) | 0.50(0.50-0.50) |
| IL-31    | 0.50(0.50-0.50) | 0.50(0.50-0.50) |
| GM-CSF   | 1.75(1.75-1.75) | 1.75(1.75-1.75) |
Abbreviations: DM, dermatomyositis; aMDA5, anti-melanoma differentiation-associated gene 5 antibodies; aTIF1γ, anti-transcription intermediary factor 1γ antibodies; MSA, myositis specific antibodies; IQR, interquartile range.

aKruskal-Wallis P<0.05,

Figures
Figure 1

Expression heatmap of DEGs identified in each GEO dataset (a) Expression heatmap of 40 DEGs (P value < 0.05 and | log FC | > 2) in DM skin dataset GSE46239. (b) Expression heatmap of 57 DEGs (P value < 0.05 and | log FC | > 2) in DM muscle dataset GSE1551. (c) Expression heatmap of 34 DEGs (P value < 0.05 and | log FC | > 2) in DM muscle dataset GSE39454.
GO and KEGG pathway analysis of DEGs in DM muscle samples. (a) Top 15 biological processes were filtered (b) 8 molecular functions were significantly enriched by overlapped
DEGs(c) 8 KEGG pathways were screened.

Figure 3

Protein-protein interaction network from DEGs in DM muscle dataset. There were 98 nodes and 1008 edges.
Figure 4

Cytokines profiling in DM. (a) Separations between MDA5 active group and HCs as well as MDA5 active and stable group were revealed by principal component analysis (PCA). (b-l) Differently expressed cytokines in DM subgroups compared with HCs. Abbreviations: aMDA5A, active group of patients positive for melanoma differentiation-associated gene 5 antibody; aMDA5S, stable group of patients positive for melanoma differentiation-associated gene 5 antibody; aTIF1γ, patients with transcription intermediary factor 1γ antibody; non MSAS, DM patients negative for myositis specific antibody; HCs, healthy controls***p<0.01, **p<0.05, *p<0.1
Figure 5

Heatmaps of cytokines profiling in DM and correlation with clinical features. (a) Cytokines classification and expression heatmap in different DM subgroups. (b) Correlation heatmap in MDA5 group. Abbreviations: aMDA5A, active group of patients positive for melanoma differentiation-associated gene 5 antibody; aMDA5S, stable group of patients positive for melanoma differentiation-associated gene 5 antibody; aTIF1γ, patients with antitranscription intermediary factor 1γ antibodies; non MSAS, DM patients negative for myositis specific antibody; HCs, healthy controls; CK, Creatine Kinase; LDH, Lactate dehydrogenase; ALT, Alanine transaminase; AST, Aspartate transaminase; CRP, C-reactive protein; ESR, Erythrocyte Sedimentation Rate; SF, Serum Ferritin; DAS, Disease Activity Score. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05
Chemokine network in DM. Interactions between DEGs correlated with chemotaxis pathway from transcriptomes analysis and cytokines upregulated from cytokinome analysis were depicted. Interactions based on lab experimental evidence were colored, including negative regulations (green), active regulations and unspecified interactions (red).

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