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Proteomics Analysis of Plasma-Derived Exosomes Unveils the Aberrant Complement and Coagulation Cascades in Dermatomyositis/Polymyositis

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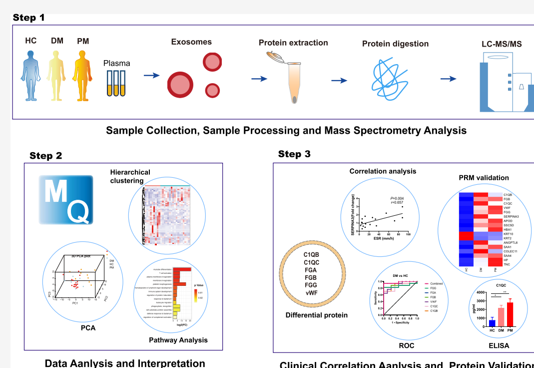
ABSTRACT: Dermatomyositis and polymyositis (DM/PM) are systemic autoimmune diseases characterized by proximal muscle weakness. The underlying pathogenetic mechanism of this disease remains under-researched. Here, using proteomics analysis, a great overlap of differentially expressed plasma exosomal proteins involved in the complement and coagulation cascade pathway, including FGA, FGB, FGG, C1QB, C1QC, and VWF, was identified in DM/PM patients versus healthy controls. Correlation analysis showed that the expression levels of complement-associated proteins (C1QB and C1QC) correlated positively with CRP, ESR, and platelet count. ROC curve analysis demonstrated that complement and coagulation cascade-associated proteins could be strong predictors for DM/PM. In addition, we also identified several other proteins that were differentially expressed in DM and PM. The selected candidate proteins were further validated by parallel reaction monitoring (PRM) and enzyme-linked immunosorbent assay (ELISA). Together, our findings indicate that these exosome-derived proteins might participate in microvascular damage in DM/PM through the activation of the complement and coagulation cascade pathway and function as biomarkers for the clinical diagnosis of DM/PM.



KEYWORDS: *dermatomyositis, polymyositis, exosomes, proteomics, complement and coagulation cascades*

■ INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) are a group of chronic autoimmune diseases defined by proximal muscle weakness and inflammation, resulting in severe mortality.[1] Dermatomyositis (DM) and polymyositis (PM) are the most common subtypes of IIMs that affect skeletal muscle with inflammatory cell infiltration, causing myofiber damage.[2] Compared with PM, DM has typical skin lesions, such as Gottron's papules and Gottron's signs.[3] Interstitial lung disease (ILD) is one of the most common complications in DM/PM that results in increased mortality.[4] Previous studies have suggested that myositis-specific antibodies (MSAs) and myositis-associated autoantibodies (MAAs) are clinically useful biomarkers to the diagnosis of IIMs and are associated with a unique clinical subset. Among these autoantibodies, anti-melanoma differentiation-associated protein 5 (anti-MDA5 antibody) is associated with DM, and anti-MDA5-positive DM patients showed severe cutaneous features, progressive interstitial lung disease (ILD), and poor prognosis.[5,6] Due to the complexity and heterogeneity of IIMs, their diagnosis and classification are often challenging.

The molecular alterations associated with the pathogenic mechanisms of IIMs remain largely unknown. To date, genome-wide association studies (GWASs) have identified multiple genetic loci as major risk factors for IIM, including major histocompatibility complexes (MHCs), TYK2, and WDFY4.[7,8] Transcriptomic profiles have demonstrated that several microRNAs' dysregulation was correlated with specific autoantibodies and clinical phenotypes in DM.[9] Notably, type I IFN, type II IFN, and IFN-induced genes are highly elevated in IIM and may serve as biomarkers for disease activity.[10−12] Increasing evidence suggests that chemokines, such as CXCL10, CXCR3, and CX3CL1, are increased in DM/PM, which might facilitate inflammatory cell infiltration into muscle tissue and other organs.[13,14] Recently, a proteomics analysis showed that Dysferlin (DYSF) is upregulated in the muscle tissues of DM/PM patients.[15]

Exosomes are a subtype of extracellular vehicles (EVs) with a diameter of 40−160 nm secreted by various cell types.[16]

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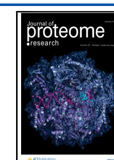

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They contain a variety of molecular components, such as DNA, RNA, lipids, metabolites, and proteins, which are equipped to mediate specific cell-to-cell communication via the transfer of their compositions to recipient cells.[17] Exosomes have been widely studied in multiple autoimmune diseases and used as biomarkers in clinical diagnosis and treatment. For example, abnormal expression of serum exosome-derived microRNAs (miRNAs) has been found in patients with SLE and lupus nephritis and correlated with disease activity.[18,19] Protein profiling revealed that plasma exosomal proteins are implicated in the pathogenesis of immunoglobulin (Ig) G4-related disease and facilitate B cell activation.[20] However, little is known about the involvement of exosomes in DM/PM.

Proteomics is a powerful technique that can obtain comprehensive protein profiles. In this study, we performed proteomic profiling of plasma-derived exosomes from DM/PM patients and healthy controls using a 4D label-free quantification (4D-LFQ) approach. We identified some differentially expressed proteins and enriched pathways by comparison of DM/PM patients and healthy controls (HCs). Expression levels of key differentially expressed proteins were further validated by parallel reaction monitoring (PRM) and enzyme-linked immunosorbent assay (ELISA). Furthermore, we investigated the correlation between abnormal protein expression and clinical features in DM/PM patients and evaluated the diagnostic potential of upregulated proteins by ROC curve analysis. Together, this study provides a comprehensive description of proteome changes of plasma-derived exosomes in patients with DM/PM and indicates the clinical relevance of exosomal proteins as potential molecular markers for diagnosis and treatment of DM/PM.

■ SUBJECTS AND METHODS

Patients and Samples

This study was approved by the Ethics Committee of the Shenzhen People's Hospital, China (LL-KY 2019514), and informed consent was obtained from all donors in the study. The present study conformed to the principles of the Declaration of Helsinki. Peripheral blood was collected in EDTA-treated collection tubes from 24 DM/PM patients and 9 healthy controls (HCs), and plasma was obtained from each peripheral blood sample and stored at −80 °C. All patients fulfilled the EULAR/ACR classification criteria for idiopathic inflammatory myopathies[21] and were enrolled at the Department of Rheumatology and Immunology, Shenzhen People's Hospital, China, from 2019 to 2021. The healthy controls were recruited from the Department of Physical Examination, Shenzhen People's Hospital. Proteomics analysis of plasma exosomes derived from 17 DM patients, 7 PM patients, and 9 healthy controls was performed. Additionally, the DM/PM patients were divided into subgroups based on whether they complicated ILD or were associated with anti-MDA5-positive antibodies. The diagnostics of ILD was based on chest high-resolution CT (HRCT). Four MDA5-positive pre- and post-treatment patients were included in DM, and methylprednisolone, cyclosporine A, and cyclophosphamide were used for the treatment of MDA5$^+$ DM patients. The clinical characteristics, types of antibodies, and laboratory data of all patients are summarized in Table S2. Antinuclear antibodies (ANAs) were detected by indirect immunofluorescence testing, and other myositis-specific antibodies (MSAs) and myositis-associated autoantibodies (MAAs) were detected by western blotting (Oumeng Medical Diagnostics, Guangzhou). In addition, the overlapping samples, including six DM patients, four PM patients, and five HCs, were used for PRM validation. Another cohort including nine patients with DM, eight patients with PM, and eight HCs was used for ELISA validation.

Exosome Extraction

One milliliter of plasma was taken from −80 °C storage and thawed quickly. The plasma was centrifuged at 1500g for 10 min to remove cells and large particles. The supernatant was collected and centrifuged at 10 000g for 30 min and then filtered through a 0.22 μm syringe membrane filter (Millipore, USA). Then, exosomes were isolated using IZON qEVS original columns (iZON Science) according to the instructions. In brief, the column was washed with eluting buffer (1 × PBS), prior to sample collection. To isolate exosomes, 500 μL of the collected supernatant was added to the qEV size-exclusion columns. The first 3 mL was considered the void volume, and then PBS was added to the separation column to elute exosomes. After passing through the void volume, high-purity exosomes were collected. The isolated exosomes were identified by transmission electron microscopy (TEM) and western blotting, and the concentration was measured by nanoparticle tracking analysis (NTA). Antibodies for CD9, CD81, TSG101, and ALIX were purchased from PTM Biolabs, and the antibody for calnexin was purchased from Abcam. Exosomes were lysed by urea buffer (8 M urea and 1% protease inhibitor) and sonicated using an ultrasonic processor. The concentration of the exosome protein was determined by the bicinchoninic acid (BCA) assay.

Liquid Chromatography−Tandem Mass Spectrometry (LC−MS)/MS Analysis

Exosomal proteins were digested into peptides by trypsin. The tryptic peptides were dissolved in mobile phase A (0.1% formic acid and 2% acetonitrile in water) of liquid chromatography and then separated using a NanoElute ultrahigh-performance liquid chromatography system. Mobile phase B was a solution containing 0.1% formic acid and 100% acetonitrile. Liquid gradient settings are as follows: 0−70 min, 6−24% B; 70−84 min, 24−35% B; 84−87 min, 35−80% B; and 87−90 min, 80% B, and the flow rate was maintained at 450 nL/min.

The peptides were separated by an ultrahigh-performance liquid system and then injected into a capillary ion source for ionization and into a timsTOF Pro mass spectrometer for analysis. The ion source voltage was set to 1.75 kV, and both the peptide precursor and its secondary fragments were detected and analyzed using high-resolution TOF MS. The MS/MS scan range was 100−1700 m/z, and data acquisition was performed in parallel accumulation serial fragmentation (PASEF) mode. Precursors with charge states of 0−5 were selected for fragmentation, and ten PASEF-MS/MS scans were acquired per cycle. The dynamic exclusion was set to 30 s.

Database Search

The MS/MS data were processed by the MaxQuant search engine (v.1.6.6.0). Tandem mass spectra were searched against the Homo_sapiens_9606 database (20366 entries) concatenated with the reverse decoy database. The method of cleavage enzyme was set to trypsin/P, the number of missing cleavages was set to 2, the minimum peptide length peptide was set to 7 amino acid residues, the mass tolerance for precursor ions was set as 20 ppm in the first search and 20 ppm in the main search, and the secondary fragment ion mass tolerance was 20

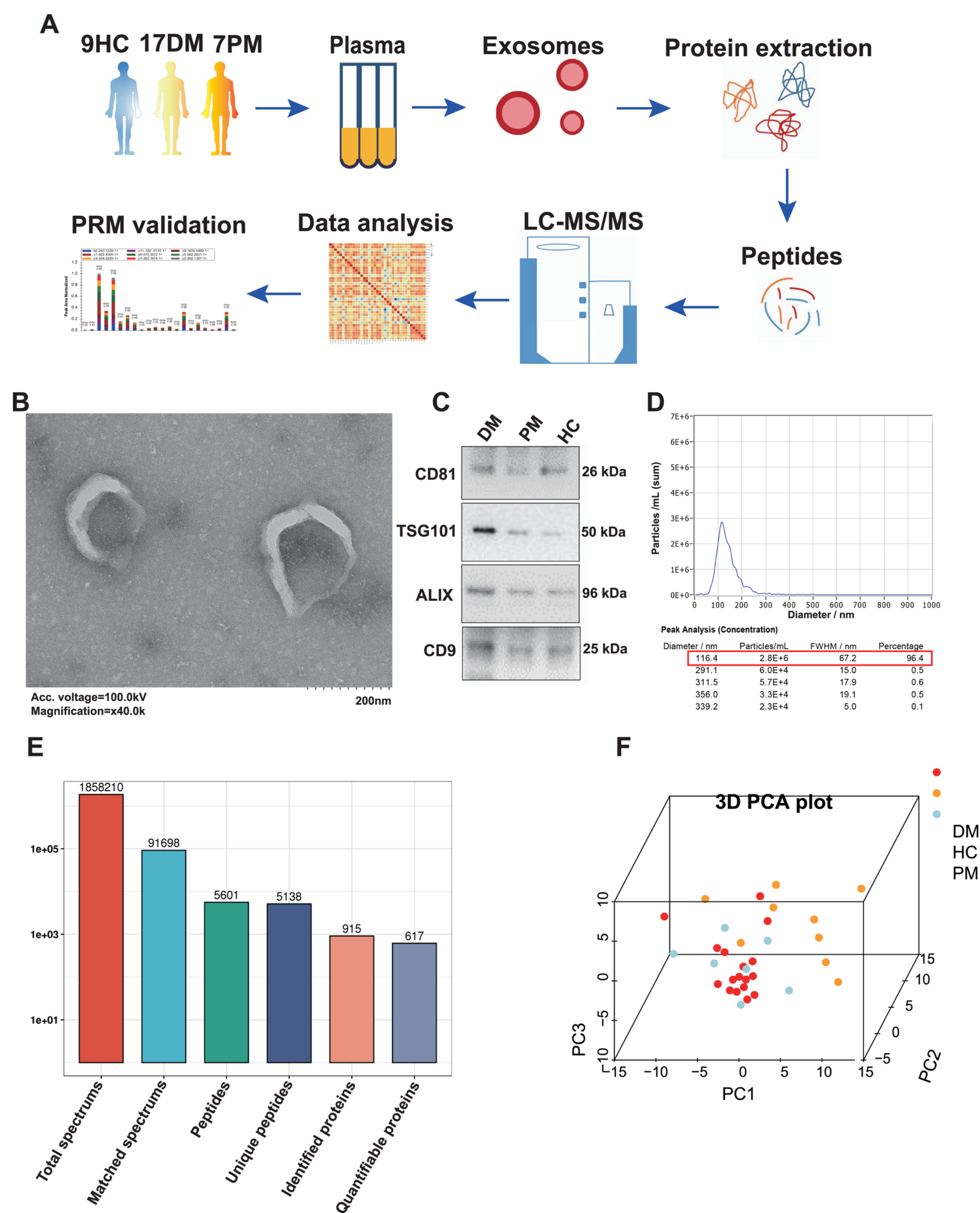

Figure 1. Schematic diagram of proteomics analysis and characterization analysis results of plasma exosomes. (A) Plasma exosomal proteins from three experimental groups, including patients with DM (n = 17), patients with PM (n = 7), and HCs (n = 9), were subjected to LC−MS/MS analysis with a 4D-LFQ approach to generate proteomic data sets and bioinformatics analysis. (B) Representative TEM micrograph of exosomes. Scale bars: 200 nm. (C) Western blots showing enrichment of exosome markers CD81, TSG101, and ALIX in exosomes. (D) Particle size and exosome concentration measured using NTA. (E) Overall proteomic statistics of plasma exosomes. (F) PCA plot of every individual sample.

ppm. Carbamidomethyl on Cys was set as a fixed modification, and ['Acetyl (Protein N-term)', 'Oxidation (M)', 'Deamidation (NQ)'] was set as a variable modification. The quantitative method was set as LFQ, and the FDR for protein identification and PSM identification was set to 1%.

Data Analysis

Proteomics analysis and PRM validation were performed with biological replicates. The relative quantitative value of each protein in the two comparison groups was tested by a t-test, and a P value < 0.05 was considered statistically significant. Enrichment analysis for Gene Ontology was classified by GO annotation into three categories: biological process (BP), cellular compartment (CC), and molecular function (MF). Enrichment analysis for KEGG was performed to identify enriched pathways. For each pathway, a two-tailed Fisher's exact test was employed to test the enrichment of the differentially modified protein against all identified proteins.

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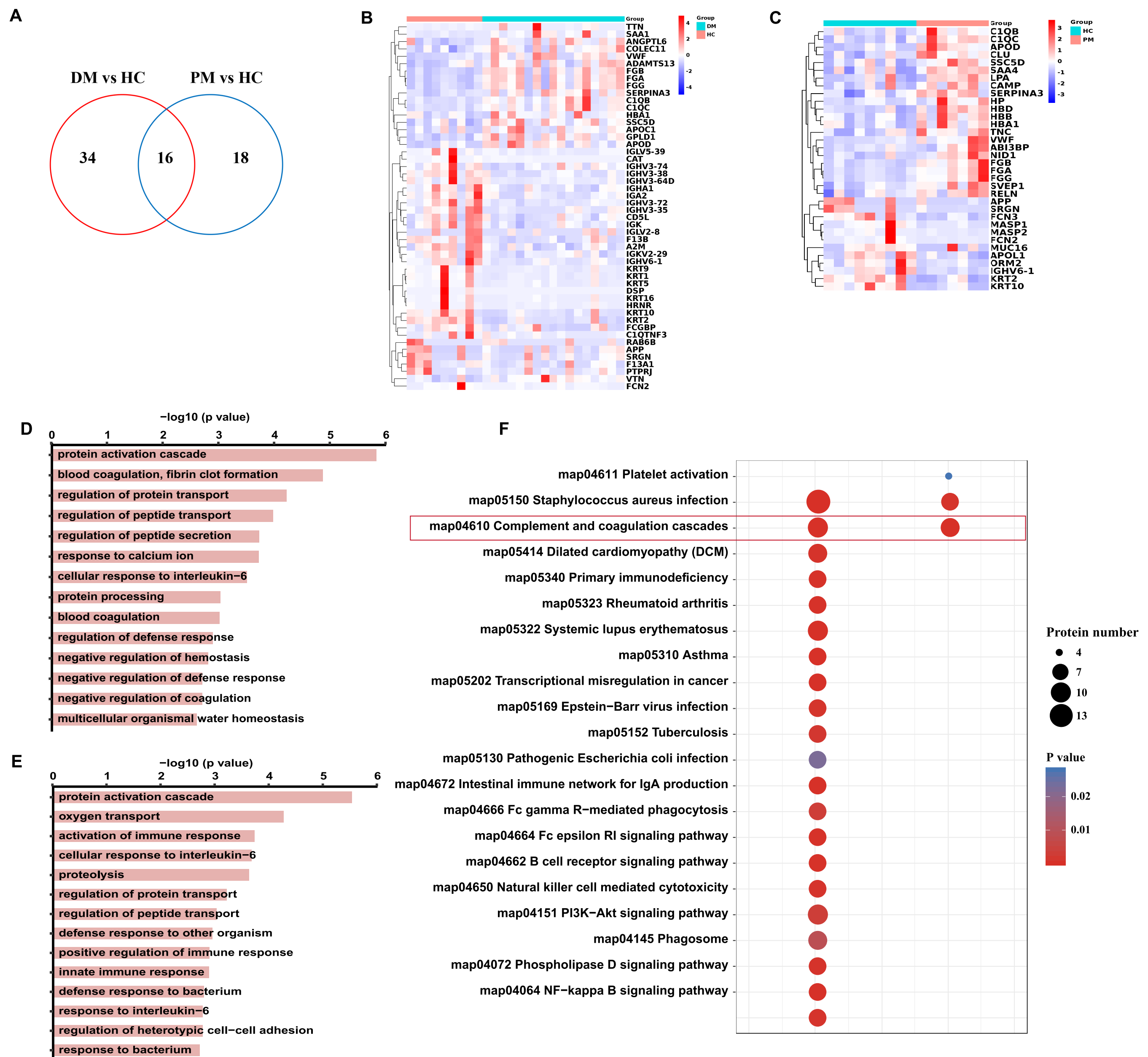

Figure 2. Differential proteomics analysis of plasma exosomes from patients with DM and PM and HCs. (A) Venn diagram summarizing the number of differential and overlapping proteins in DM and PM. (B, C) Unsupervised clustering analysis showing differentially expressed proteins in patients with DM and PM compared with HCs. Differentially expressed proteins were considered using *P* value < 0.05 and FC > 1.5, color-coded as red for upregulated and blue for downregulated. (D, E) Top-ranked GO (biological processes) enrichment terms for differentially expressed proteins in DM and PM (*P* value < 0.05). Differentially expressed proteins were considered using *P* value < 0.05 and FC > 1.5. (F) Dot plot representing significantly enriched KEGG pathways of differentially expressed proteins in DM and PM (*P* value < 0.05). Differentially expressed proteins were considered using *P* value < 0.05 and FC > 1.5.

The GO and KEGG pathways were considered significantly enriched with the corrected *P* value < 0.05.

PRM Validation

The proteins identified were selected for PRM validation based on the sequencing data. The tryptic peptides were dissolved in 0.1% formic acid (solvent A) and then separated on an EASY-nLC 1000 high-performance liquid chromatography (HPLC) system. After that, the peptides were subjected to an NSI source followed by tandem mass spectrometry (MS/MS) in a Q Exactive^{TM} Plus (Thermo) coupled online to the UPLC system. The acquired MS data were processed using Skyline (v.21.1). The enzyme was set as trypsin [KR/P], and the max missed cleavage was set as 0. The peptide length was set as 7−25. Carbamidomethyl on cysteine and oxidation on Met were set as variable modifications, and the number of max variable modifications was set as 3. The transition settings are as follows: precursor charges were set as 2 and 3, ion charges were set as 1, and ion types were set as b and y. The product ions were set from ion 3 to the last ion, and the ion match tolerance was set as 0.02 Da. Each peptide was quantified by comparing the fragment ion peak area to its corresponding transitions, and statistical significance was defined as a *P* value < 0.05 using a two-tailed Student's *t*-test.

ELISA Validation

ELISA was performed for proteins identified in proteomics analysis. The expression levels of C1QC (Reddot Biotech), FGA (Reddot Biotech), FGB (Reddot Biotech), and VWF (Reddot Biotech) were measured by ELISA according to the instructions provided by the kits.

Statistical Analysis

All data analyses were performed by SPSS version 25.0 and GraphPad Prism software version 8.0. Two-tailed Student's *t*-test was used to analyze differences between the DM/PM and HC groups, and a P value < 0.05 was considered to denote a significant difference. Correlation analysis between protein expression values and clinical examinations was performed by Spearman's correlation analysis, and a P value < 0.05 was considered statistically significant. The ROC curve was used to evaluate the diagnostic performance of each protein. The area under the receiver operating curve (ROC), cutoff, sensitivity, and specificity were calculated by SPSS, and a P value < 0.05 was considered statistically significant.

RESULTS

Characterization of Plasma-Derived Exosomes in DM/PM Patients and HCs

To perform a comprehensive proteomics analysis of plasma-derived exosomes from patients with DM/PM and healthy controls, we collected plasma samples from 17 patients with DM, 7 patients with PM, and 9 HCs and purified exosomes from these plasma samples. The proteins were extracted from exosomes and digested into peptides with trypsin. Then, proteomics analysis was performed with a label-free quantification approach by liquid chromatography−tandem mass spectrometry (LC−MS/MS) (Figure 1A). In addition, transmission electron microscopy (TEM) was used to confirm the presence and size of isolated exosome particles (Figure 1B). Western blot analysis showed that the vesicles were positive for exosome markers CD81, ALIX, TSG101, and CD9 (Figures 1C and S1). In addition, we detected calnexin by WB. Calnexin (negative marker), an endoplasmic reticulum-specific protein, was absent in the isolated particles (Figure S3). Nanoparticle tracking analysis (NTA) was used to determine the size and concentration of exosome particles, and the results showed that the isolated particles had the highest percentage (96.4%) with a size of 116.4 nm (Figure 1D). After data processing, 915 proteins were found in the proteome, and a total of 617 proteins were quantified across all samples (Figure 1E, Table S1). PCA showed a clear distinction between DM patients and HCs or PM patients and HCs, while there was an overlap between DM and PM patients (Figure 1F).

Proteomic Profiling of Plasma-Derived Exosomes

Then, the differentially expressed proteins in plasma-derived exosomes of DM/PM patients were analyzed, and a total of 50 and 34 differentially expressed proteins (DEPs) were found in the DM and PM patients, respectively. Of these differentially expressed proteins, 16 proteins were common to both groups. (Figure 2A and Table 1). The heatmap showed all DEPs in patients with DM (Figure 2B) and PM (Figure 2C) (fold change > 1.5, and P value < 0.05). To better elucidate the biological function of DEPs, GO Ontology and KEGG pathway enrichment analysis were conducted. Biological process analysis revealed some common functions for DEPs in DM and PM, such as the protein activation cascade, regulation of protein transport, regulation of peptide transport, and cellular response to interleukin-6 (Figure 2D,E). In addition, KEGG pathway analysis revealed that complement and coagulation cascades, mainly regulated by FGA, FGB, FGG, C1QB, C1QC, VWF, A2M, and F13, were significantly enriched in both DM and PM groups, and the NF-kappa B signaling pathway, B cell receptor signaling pathway, and Fc epsilon RI signaling pathway were specifically enriched in DM (Figure 2F). Taken together, these results revealed distinct protein profiles and dysregulated pathways in DM/PM patients compared with HCs.

Table 1. Common Regulated Proteins in Exosomes of DM and PM Patients

protein	fold change: DM vs HC	P value	fold change: PM vs HC	P value	regulated type
C1QB	5.1645	0.0005	2.6824	0.0186	up
C1QC	3.6376	0.0011	2.3475	0.0092	up
VWF	3.405	0.0006	4.9249	0.0025	up
FGA	3.1354	0.0132	4.0887	0.0003	up
FGB	3.8793	0.004	4.2536	0.0002	up
FGG	3.0321	0.0143	3.898	0.0003	up
SERPINA3	2.1131	0.0064	1.5638	0.015	up
HBA1	1.8143	0.0448	2.4847	0.0362	up
APOD	1.6502	0.0042	2.0031	0.0006	up
SSC5D	1.6928	0.004	1.7803	0.0262	up
KRT2	0.4231	0.00005	0.4778	0.0043	down
KRT10	0.4412	0.0008	0.4673	0.0174	down
APP	0.4404	0.0171	0.3838	0.0355	down
IGHV6-1	0.3178	0.0148	0.2162	0.0176	down
SRGN	0.3251	0.0227	0.1877	0.042	down
FCN2	0.144	0.0464	0.087	0.0475	down

Differentially Expressed Proteins in Plasma-Derived Exosomes from DM/PM Subgroups

We next sought to identify the DEPs of plasma-derived exosomes in patients with DM/PM complicated with ILD. All 24 patients with DM/PM were enrolled; of them, 15 patients were complicated by ILD (DM/PM_ILD$^+$ patients) and 9 patients were uncomplicated by ILD (DM/PM_ILD$^-$ patients). Then, the DEPs were identified, and the GO enrichment analysis was conducted. As shown in Figure 3A, the results of GO analysis indicated that the top-enriched biological processes were mainly related to myotube differentiation, T cell activation, and plasma membrane invagination. We also investigated the DEPs of plasma-derived exosomes in anti-MDA5 antibody-positive DM patients compared to anti-MDA5 antibody-negative DM patients. A total of 17 DM patients were enrolled; of them, 11 were anti-MDA5 antibody-positive DM patients (MDA5$^+$ DM patients) and 6 were anti-MDA5 antibody-negative DM patients (MDA5$^-$ DM patients). Then, the DEPs were identified. Among these DEPs, SERPINA3 was highly expressed in MDA5$^+$ DM patients (Figure 3B) and also found to be highly expressed in DM and PM patients (Figure 2B). GO analysis of DEPs in MDA5$^+$ DM patients revealed that the enriched GO terms for biological processes were mainly associated with skin development, intermediate filament cytoskeleton organization, and epithelial cell differentiation. Further analysis was performed to identify the DEPs in MDA5$^+$ DM patients complicated with ILD (MDA5_ILD$^+$ patients; n = 8) compared to MDA5$^+$ DM patients uncomplicated with ILD

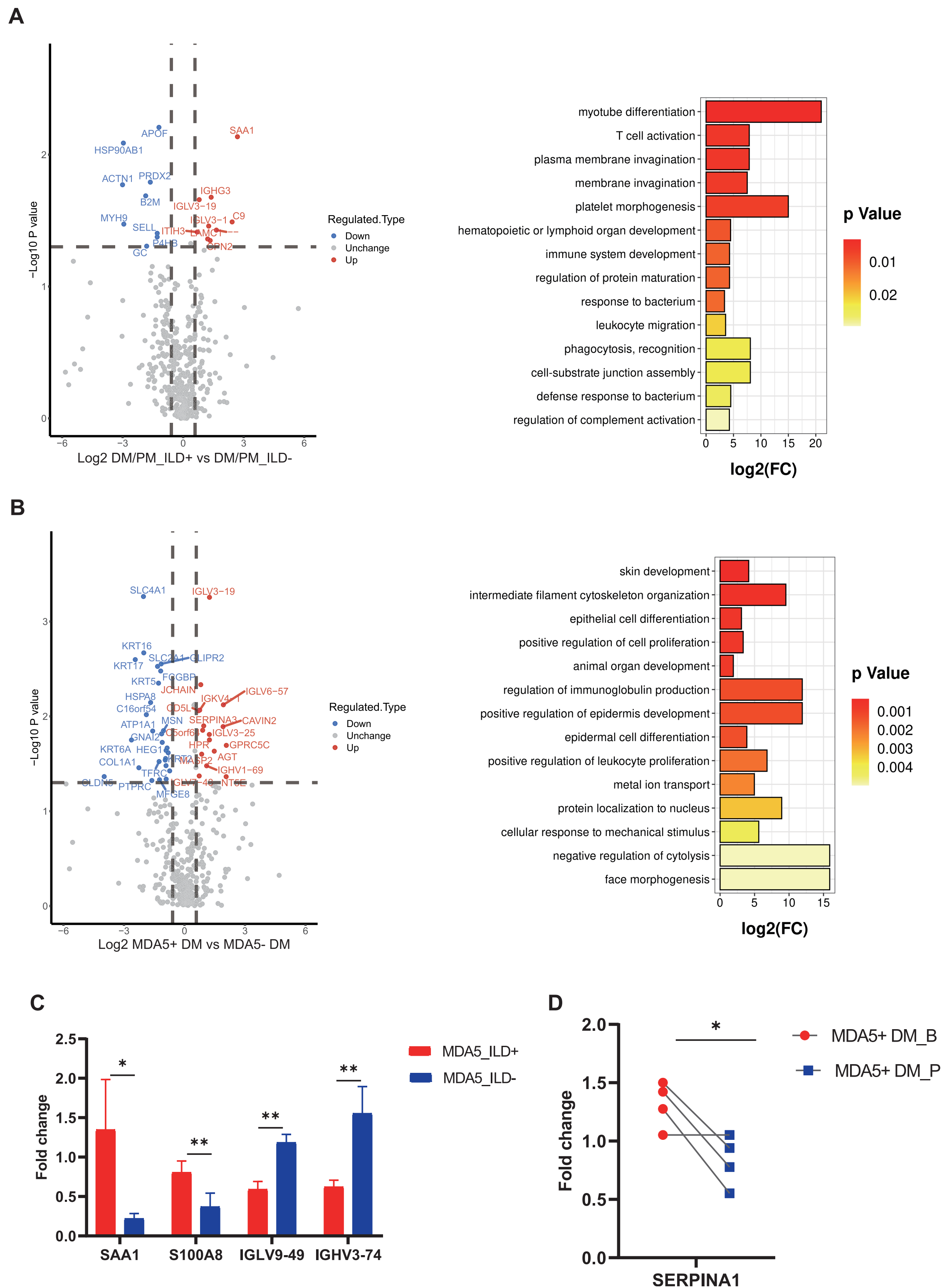

Figure 3. Differentially expressed plasma exosomal proteins in DM/PM subtypes. (A, B) Left: volcano plots comparing DM/PM_ILD$^+$ patients (n = 15) with DM/PM_ILD$^-$ patients (n = 9) and comparing MDA5_P patients (n = 11) with MDA5_N patients (n = 6), as indicated. Significantly DEPs are highlighted in red (up) and blue (down). P < 0.05 and FC > 1.5 were considered significant. Right: enrichment analysis using Gene Ontology (GO) annotations showing the top GO terms based on biological processes. (C) DEPs analysis in MDA5_ILD$^+$ patients (n = 8) compared to MDA5_ILD$^-$ patients (n = 3) (P value < 0.05, FC > 1.5). (D) Difference in SERPINA1 between pretreatment (n = 4) and post-treatment (n = 4) (P value < 0.05, FC > 1.5).

(MDA5_ILD$^-$ patients; n = 3). Our results showed that SAA1 and S100A8 were significantly increased in MDA5_ILD$^+$ patients (Figure 3C). S100A8 has been previously reported to be associated with ILD development in DM patients.[22] In addition, we conducted a comparative analysis of pre- and post-treatment MDA5$^+$ DM patients with immunosuppressant

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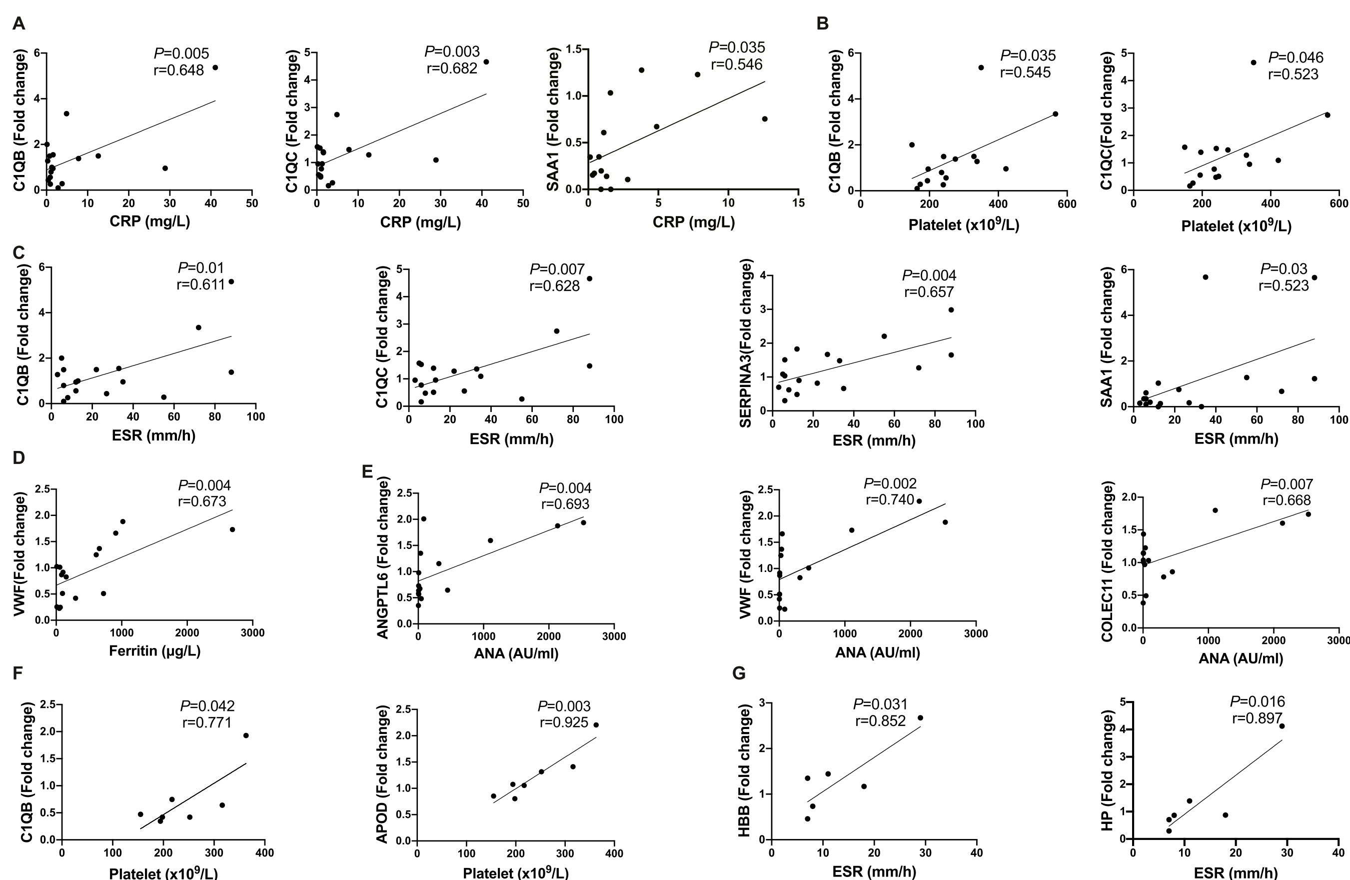

Figure 4. Correlations between the protein expression level of plasma exosomes and clinical features. (A−E) Correlations between the protein expression levels of plasma exosomes and the clinical features of DM. (F, G) Correlations between the protein expression levels of plasma exosomes and clinical features of PM. Correlations were tested by Pearson's correlation coefficient r and P value, and a P value < 0.05 was considered significant.

drugs. Our results showed that SERPINA1 was significantly downregulated in three out of four post-treatment patients (Figure 3D). Together, our results suggest distinct molecular features in different DM/PM subtypes.

Correlation between the Expression Levels of Plasma Exosomal Proteins and Clinal Features in DM/PM Patients

To investigate whether the differentially expressed exosomal proteins are correlated with the clinical features of DM/PM patients, we evaluated the correlation between the protein expression levels and creatine kinase (CK), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet, ferritin, antinuclear antibody (ANA), and IgG. We found that several upregulated proteins correlate significantly with the clinical features of patients with DM/PM. Complement-associated proteins (C1QB and C1QC) were found to be positively correlated with CRP (P = 0.005, r = 0.648, and P = 0.003, r = 0.682) (Figure 4A), platelet (P = 0.035, r = 0.545 and P = 0.046, r = 0.523) (Figure 4B), and ESR (P = 0.010, r = 0.611 and P = 0.007, r = 0.628) (Figure 4C) in DM. C1QB was also found to be positively correlated with platelets (P = 0.0442, r = 0.771) (Figure 4F) in PM. von Willebrand factor (VWF) correlated positively with ferritin (P = 0.004, r = 0.673) (Figure 4D) and ANA (P = 0.002, r = 0.740) (Figure 4E) in DM. VWF is a large multimeric glycoprotein that is cleaved by ADAMTS13, mediating platelet adhesion to injured vessel walls. We found that ADAMTS13 was also positively correlated with ANA (P = 0.021, r = 0.590) (Figure 4E) in DM. Acute-phase proteins, including SAA1 (serum amyloid A-1 protein), HP (haptoglobin), and SERPINA3 (α-1-antichymotrypsin), also correlated with clinical parameters. Of these, SAA1 positively correlated with CRP and ESR (P = 0.035, r = 0.546 and P = 0.030, r = 0.523) (Figure 4A,C) in DM. SERPINA3 positively correlated with ESR (P = 0.004, r = 0.657) (Figure 4C) in DM, and HP positively correlated with ESR (P = 0.016, r = 0.897) (Figure 4G) in PM, while there were no significant correlations between these upregulated proteins and CK. Together, these results suggested that the upregulation of these proteins promote the development and severity of the disease.

Prediction of Diagnostic Ability of Differential Protein Expression in Plasma Exosomes from DM/PM Patients

To assess the diagnostic potential of these differentially expressed plasma exosomal proteins in DM/PM patients, we performed a ROC curve analysis of the upregulated proteins. The ROC curve (AUC) was calculated for each protein, and our results showed that 15 proteins had high AUC values in DM patients compared with HCs (P < 0.05) (Figure 5A). Among these proteins, complement and coagulation cascade pathway-associated proteins, including C1QB, C1QC, VWF, FGA, FGB, and FGG, had higher AUCs. When combining these complement and coagulation cascade pathway-associated proteins, the AUC was increased to 0.974, while the sensitivity and specificity were 94.1 and 100%, respectively (Figure 5C and Table 2). Other proteins also had the ability to

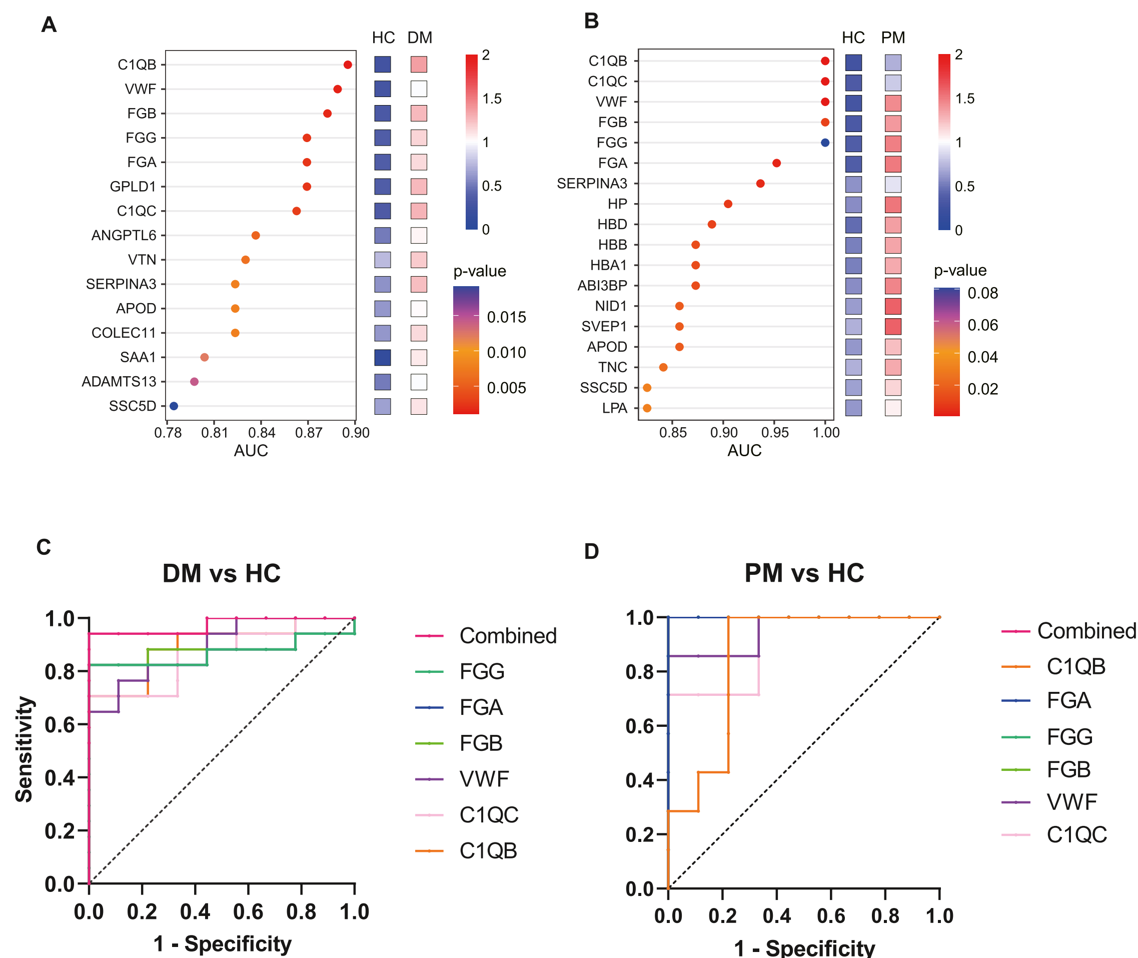

Figure 5. ROC curve analysis of upregulated plasma exosomal proteins in DM/PM. (A, B) AUC values and fold change of protein expression are shown in DM and PM, respectively; a P value < 0.05 was considered significant. (C, D) ROC curves for the six-protein signature overlaid with each protein showing the diagnostic capacity of DM and PM.

Table 2. Diagnostic Performances of Upregulated Proteins in DM Patients[a]

protein name	AUC	(95% CI)	P value	sensitivity	specificity	cutoff value
C1QB	0.8954	0.7732−1.000	0.0011	0.7060	1.0000	0.7450
VWF	0.8889	0.7656−1.000	0.0013	0.7650	0.8890	0.4910
FGB	0.8824	0.7410−1.000	0.0016	0.8240	1.0000	0.6590
FGG	0.8693	0.7224−1.000	0.0023	0.8240	1.0000	0.7440
FGA	0.8693	0.7224−1.000	0.0023	0.8240	1.0000	0.6730
GPLD1	0.8693	0.7309−1.000	0.0023	0.7060	0.8890	0.6960
C1QC	0.8627	0.7232−1.000	0.0028	0.7060	1.0000	0.7050
ANGPTL6	0.8366	0.6454−1.000	0.0055	0.9410	0.6670	0.4680
VTN	0.8301	0.6302−1.000	0.0065	0.7650	0.8890	0.9880
SERPINA3	0.8235	0.6638−0.9833	0.0076	0.6880	1.0000	0.8140
APOD	0.8235	0.6623−0.9848	0.0076	0.8820	0.6670	0.5990
COLEC11	0.8235	0.6654−0.9817	0.0076	0.6470	1.0000	1.0070
SAA1	0.8039	0.6275−0.9803	0.0122	0.8240	0.7780	0.1320
ADAMTS13	0.7974	0.6204−0.9743	0.0142	0.8820	0.6670	0.4690
SSC5D	0.7843	0.5870−0.9816	0.0191	1.0000	0.5560	0.5260
combined	0.9740	0.9170−1.0000	0.0001	0.9410	1.0000	0.6700

[a]Combined: including C1QB, C1QC, VWF, FGA, FGB, and FGG.

discriminate DM patients from HCs, such as GPLD1, ANGPTL6, VTN, SERPINA3, APOD, and COLEC11 (Figure 5A and Table 2). Similar results were found in the PM groups compared with the HC group, and complement and coagulation cascade pathway-associated proteins showed higher AUCs (AUC = 1) than other proteins. The AUC of the combination of these complement and coagulation cascade pathway-associated proteins also reached 1, and the sensitivity and specificity were 100 and 100%, respectively (Figure 5D and Table 3). We validated some plasma exosomal proteins by

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Table 3. Diagnostic Performances of Upregulated Proteins in PM[a]

protein name	AUC	(95% Cl)	P- value	sensitivity	specificity	cutoff value
C1QB	1.0000	1.000−1.000	0.0009	1.0000	0.7780	0.3060
C1QC	1.0000	1.000−1.000	0.0009	0.7140	1.0000	0.6790
VWF	1.0000	1.000−1.000	0.0009	0.8570	1.0000	0.6170
FGB	1.0000	0.7024−1.000	0.0095	1.0000	1.0000	0.6460
FGG	1.0000	0.5043−1.000	0.0807	1.0000	1.0000	0.7270
FGA	0.9524	0.8472−1.000	0.0026	1.0000	1.0000	0.6710
SERPINA3	0.9365	0.8194−1.000	0.0036	0.8570	0.8890	0.7600
HP	0.9048	0.7533−1.000	0.0070	0.8570	0.6670	0.6900
HBD	0.8889	0.7282−1.000	0.0095	0.8570	0.8890	0.8500
HBB	0.8730	0.6799−1.000	0.0129	0.7140	1.0000	1.0310
HBA1	0.8730	0.6907−1.000	0.0129	0.5710	1.0000	1.1170
ABI3BP	0.8730	0.6797−1.000	0.0129	0.7140	0.8890	0.7940
NID1	0.8571	0.6605−1.000	0.0172	1.0000	0.6670	0.5950
SVEP1	0.8571	0.6586−1.000	0.0172	0.7140	1.0000	1.1690
APOD	0.8571	0.6586−1.000	0.0172	1.0000	0.7780	0.7320
TNC	0.8413	0.6441−1.000	0.0229	1.0000	0.5560	0.6630
SSC5D	0.8254	0.6159−1.000	0.0300	1.0000	0.4444	0.5910
LPA	0.8254	0.6208−1.000	0.0300	0.8571	0.8890	1.0045
combined	1.0000	1.0000−1.0000	0.0001	1.0000	1.0000	0.5000

[a]Combined: including C1QB, C1QC, VWF, FGA, FGB, and FGG.

ELISA in another cohort and performed ROC curve analysis. We also found that C1QC, FGA, FGB, and VWF had a high AUC in patients with DM and PM compared with HCs (Figure S2). Several other differentially expressed proteins, including SERPINA3, HP, HBD, HBA1, and ABI3BP, also had discriminative values between PM patients and HCs (Figure 5B and Table 3). Together, these results demonstrated that these differentially expressed exosomal proteins have a diagnostic value in distinguishing DM or PM patients from HCs.

Validation of Differential Protein Expression in Plasma Exosomes from DM/PM Patients

To validate the plasma exosomal proteins identified in the discovery cohort, we performed PRM assays. PRM is a targeted mass spectrometry approach that can achieve relative or absolute quantification of the target protein by selectively detecting specific or target peptides.[23] Using PRM, we quantified 17 plasma exosomal proteins, and the expression levels of most proteins were consistent with the 4D-LFQ results (Figure 6A). Our results indicated that the expression levels of C1QB, C1QC, FGB, FGG, VWF, SERPINA3, APOD, and SSC5D were higher in both DM and PM patients than in HCs, and the difference of these proteins in DM patients was significant, while the expression levels of C1QB, FGB, FGG, and APOD were significantly different in PM patients (Figure 6B). Our results also showed that the expression levels of ANGPT16, SAA1, and COLEC11 were significantly higher in DM patients than in HCs. In addition, the expression levels of HP and TNC were higher in PM patients than in HCs, whereas the expression levels of HP were significantly different in PM patients (Figure 6B). Furthermore, we also validate some plasma exosomal proteins by ELISA in another cohort. The result indicated that C1QC, FGA, FGB, and VWF were highly expressed in DM and PM patients compared to HCs. Among them, the expression levels of C1QC, FGA, and VWF were significantly upregulated (Figure 6C).

■ DISCUSSION

This study provides the first proteomic characterization of plasma-derived exosomes from DM/PM patients and healthy controls. Exosomes are cell-derived microvesicles that are implicated in intercellular communication, enabling the functional transfer of their cargo (proteins, lipids, and RNAs) between local or distant cells. Accumulating evidence has demonstrated that exosomes are implicated in physiological and pathological processes in various diseases and have been recognized as valuable diagnostic and prognostic biomarkers.[24,25] In this study, we analyzed the protein profiles of plasma-derived exosomes of DM/PM patients to identify potential exosome proteins as diagnostic biomarkers for patients with DM or PM. Overall, 50 DEPs were identified in patients with DM compared with HCs, and 34 DEPs were identified in patients with PM compared with HCs. Among these DEPs, 16 DEPs were common to both DM and PM patients. GO function and KEGG pathway enrichment analysis also showed that the dysregulation of exosome proteins identified in both DM and PM patients is significantly enriched in the complement and coagulation cascade pathway.

The complement system is a major component of innate immunity and mainly participates in host defense.[26] However, the dysregulation of the complement system can lead to tissue damage, pathological inflammation, and activation of the coagulation pathway. A series of studies showed that inappropriate complement activation and complement deficiencies were involved in the pathogenesis and clinical manifestations of several autoimmune diseases, such as systemic lupus erythematosus (SLE),[27] vasculitides,[28] and rheumatoid arthritis (RA).[29] In DM/PM, several previous studies suggested that complement-mediated microvasculopathy is a pathogenic feature of dermatomyositis. The binding of C1q to injured endothelial cells triggered the activation of the classical complement pathway, resulting in microvascular membrane attack complex (MAC) deposits in dermatomyositis. The deposition of MAC on intramuscular capillaries led to the depletion or loss of microvasculature and capillaries, resulting in sequential ischemic muscle fiber damage,

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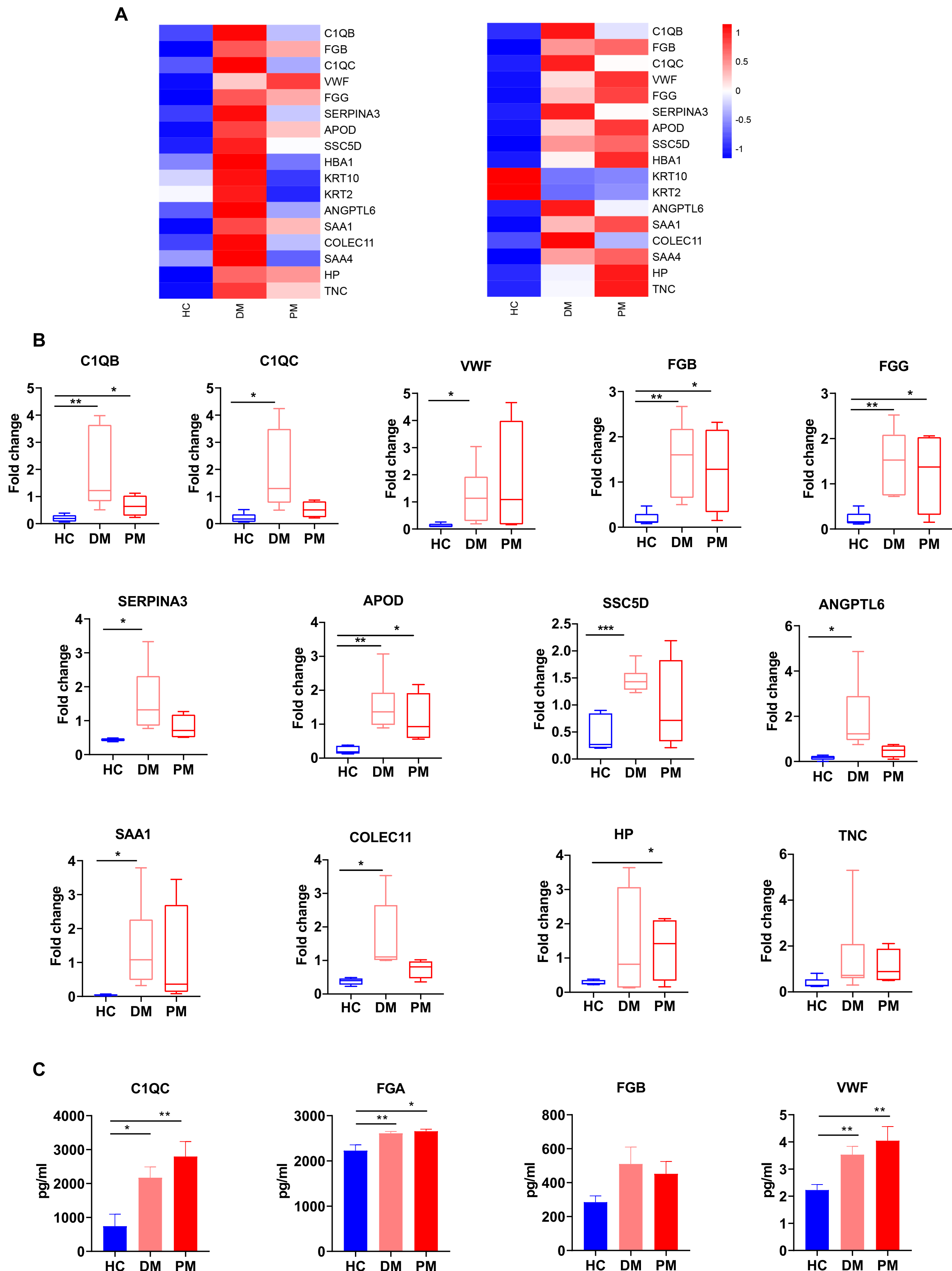

Figure 6. Parallel reaction monitoring (PRM) validation of plasma exosomal proteins. (A) Heatmap summarizing 17 quantified plasma exosomal proteins. The PRM (left) and 4D-LFQ data (right) were depicted, and plasma exosomal proteins were clustered according to the PRM profiles and 4D-LFQ data. (B) Validated plasma exosomal proteins by PRM showing the same trend as 4D-LFQ. Note: *, **, and *** represent P values < 0.05, < 0.01, and < 0.001, respectively. (C) Expression levels of plasma exosomal proteins including C1QC, FGA, FGB, and VWF in patients with DM ($n = 9$), patients with PM ($n = 9$) and healthy controls (HCs) ($n = 8$) determined by ELISA. Note: *, **, and *** represent P values < 0.05, < 0.01, and < 0.001, respectively.

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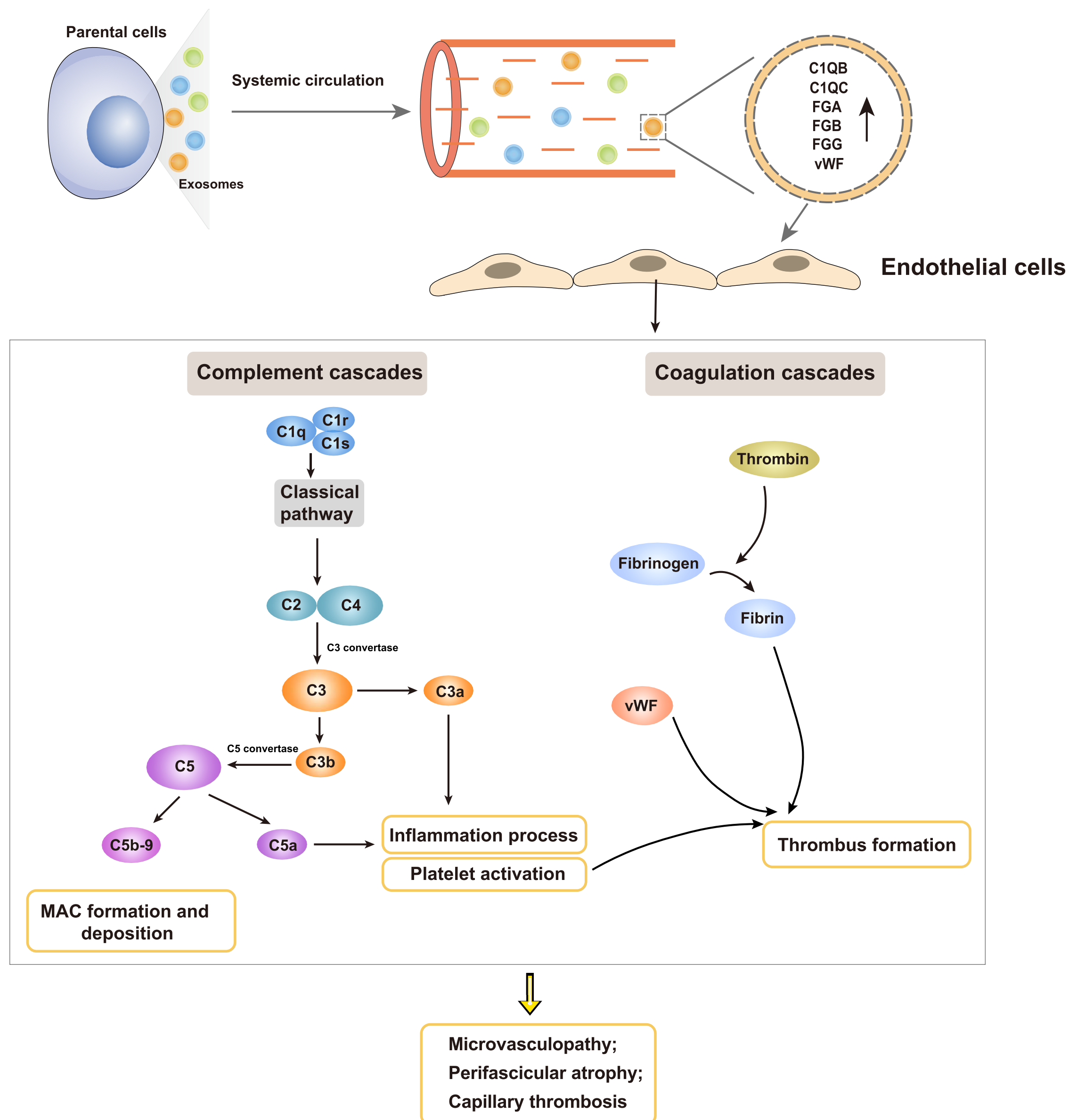

Figure 7. Schematic representation of the proposed mechanism by which plasma-derived exosomes use the complement and coagulation system to induce the lysis of vascular endothelial cells in DM/PM. C1q deposition on vascular endothelial cells induces the activation of classical pathway and increases expression of C2, C4, C3a/C3b, C5a/C5b, and C5b9/MAC in endothelial cells, resulting in the death of these endothelial cells. Meanwhile, products C3a and C5a also play key roles in inflammation, platelet activation, and the recruitment of adaptive immune cells. FGA, FGB, and FGG are associated with the coagulation cascade. VWF promotes the recruitment and adhesion of activated platelets at the injury site and carries factor VIII to the platelet surface to support generation of thrombin. Thrombin converts fibrinogen to fibrin, which contributes to the formation and stabilization of microvascular thrombosis.

perifascicular atrophy, and muscle weakness.[30−32] In addition, an early study showed that serum C1q concentrations were increased in PM/DM patients with elevated ESR and have been used as potential serological markers.[33] Complement component C4d has been shown to be expressed in the perimysial vessels and endomysial capillaries and used as a marker of complement activation for the diagnosis of DM.[34] In our study, proteomics analysis of plasma-derived exosomes showed that complement components C1QB and C1QC were significantly higher in DM/PM patients compared to HCs. Correlation analysis revealed that the expression levels of complement-associated proteins (C1QB and C1QC) correlated positively with disease activity. ROC curve analysis also suggested that complement-associated proteins presented diagnostic performance with higher sensitivity and specificity. In addition, several studies have shown that the interaction of the complement system and interferon pathway perpetuates vascular injury.[35−37] Also, both complement-dependent microangiopathy and activation of the type I IFN pathway have been shown to play major roles in DM.[38,39] A model of the interferon-complement loop was proposed in transplant-associated thrombotic microangiopathy that contributes to perpetuating vascular endothelial damage and thrombotic microangiopathy. In this model, interferons promote the expression of C1q, resulting in the activation of the classical complement pathway and the formation of MAC and endothelial damage. Conversely, the production of complement C5a receptor can maintain interferon secretion and

sustain endothelial damage.[40] Based on these previous studies and our results, the possible mechanisms of plasma-derived exosomes involved in the pathogenesis of patients with DM/PM were proposed. We speculated that plasma-derived exosomes with elevated C1q components (C1QB and C1QC) were delivered to intramuscular capillaries and deposited on endothelial cells, resulting in aberrant complement activation and MAC deposits and mediating the apoptosis of endothelial cells. Furthermore, the activation of the complement cascade triggered the production of proinflammation cytokines and chemokines, leading to the recruitment of immune cells and continual activation of the complement system (Figure 7).

Our results also revealed that the expression of coagulation-associated proteins (FGA, FGB, FGC, and VWF) was significantly higher in DM/PM patients compared to HCs. Accumulating evidence has suggested that the interaction of coagulation factors was involved in tissue repair and inflammatory responses in inflammatory diseases.[38] FGA, FGG, and FGB genes encode for fibrinogen α, fibrinogen β, and fibrinogen γ chains, respectively. Fibrinogen is a critical component of the coagulation cascade that is cleaved into fibrin to stabilize the formation of a blood clot. Previous studies showed that fibrinogen in plasma extracellular vesicles contributes to the perpetuation of neuroinflammation and relapses in neuroinflammatory diseases. The activation of the coagulation cascade converts fibrinogen to proinflammatory fibrin, which is deposited in active and chronic MS lesions, inducing demyelination, and autoimmune responses in neuroinflammatory diseases.[41,42] The von Willebrand factor (VWF) is a large adhesive glycoprotein that plays an important role in hemostasis and thrombosis. They facilitate the recruitment and adhesion of platelets at the site of vessel injury and contribute to the fibrin clot formation through binding to coagulation factor VIII.[43] The interaction of VWF and platelets is critical in microvascular thrombosis. In anti-MDA5 DM patients, a recent study found that VWF released by damaged endothelial cells leads to coagulation activation, vascular fibrin deposit, and thrombosis.[44] Vascular fibrin deposition and endothelial injury are characteristic pathogenic changes of DM.[6,45] Therefore, we hypothesized that elevated concentrations of fibrinogen, factor VIII, and von Willebrand factor (VWF) in plasma exosomes lead to vascular fibrin deposition, endothelial injury, and small-vessel vasculitis and capillary thrombosis in DM/PM patients (Figure 7). Furthermore, the predictive power of these dysregulated proteins was assessed by ROC analysis. We found that complement and coagulation cascade pathway-associated proteins, including C1QB, C1QC, VWF, FGA, FGB, and FGG, presented higher AUCs with sufficient sensitivity and specificity in DM/PM patients than in HCs, and the AUC was close to 1, either individually or in combination. Therefore, these coagulation cascade pathway-associated proteins are expected to be promising biomarkers of DM/PM.

In addition, the expression of SERPINA3, APOD, and SSC5D was also higher in patients with DM/PM compared to HCs. SERPINA3 is a member of the serine protease inhibitors of the serpin superfamily. Several studies have demonstrated SERPINA3's involvement in various biological activities, including inflammatory response,[46] complement system activation,[47] and apoptosis.[48] SERPINA3 has been reported in a variety of autoimmune diseases. Turnier et al.[49] suggested that urine SERPINA3 is a potential biomarker for lupus nephritis activity. Fissolo et al.[50] displayed that elevated SERPINA3 in the cerebrospinal fluid may be used as a biomarker for progressive forms of primary progressive multiple sclerosis (MS).

Then, we analyzed the exosome DEPs in DM/PM_ILD$^+$ patients compared with DM/PM_ILD$^-$ patients. Our results found that the expression of SAA1, C9, CPN2, and LAMC1 was higher in DM/PM_ILD$^+$ patients compared to DM/PM_ILD$^-$ patients. Serum amyloid A (SAA) is an acute-phase protein that shares many similarities with C-reactive protein (CRP) and plays an important role in the pathogenesis of the inflammatory rheumatic disease.[51] It has been reported that SAA proteins regulate the cytokine production of Th17 cells and promote Th17-mediated inflammatory diseases.[52] Increased serum concentrations of SAA1 and SAA2 were found in various Th17-mediated autoimmune diseases, including MS, RA, and Crohn's disease (CD).[53] Leung et al.[54] found that the expression of SAA1.2, an SAA1 protein isoforms, plays an important role in the initiation and progression of RA and can be used in the early diagnosis and severity prediction of RA. Component 9 (C9) is involved in the final stage of complement activation, which forms a membrane attack complex (MAC) during the membrane attack stage to dissolve target cells.[55] We speculated that it might be delivered to target cells by plasma-derived exosomes, promoting classical pathway activation and mediating tissue inflammation and damage in DM/PM_ILD$^+$ patients. Our results also revealed that the expression of MASP2 was higher in MDA5$^+$ DM patients compared to MDA5$^-$ DM patients. Mannose-binding lectin-associated serine protease (MASP2) is a serine protease molecule that is involved in complement activation. MASP2 can be used as a biomarker for SLE, and the high serum MASP2 levels are correlated with laboratory characteristics (nephritis, arthritis, anti-dsDNA antibody). The research also revealed that the MASP2 gene polymorphisms related to SLE genetic susceptibility in a Han Chinese population.[56] In addition, we also found that the expression of SERPINA1 was significantly downregulated in post-treatment MDA5$^+$DM patients compared to pretreatment MDA5$^+$DM patients. The serine protease inhibitor-A1 (SERPINA1) gene that encodes α-1-antitrypsin (ATT) is another member of the serpin superfamily. ATT, an acute-phase protein, exhibits inhibitory capacity against serine protease, neutrophil elastase, and other enzymes in inflammatory cells.[57,58] It is reported that the concentration of ATT was rapidly increased in response to inflammatory processes.[59] In SLE, Aggarwal et al. showed that urinary ATT might be a potential biomarker of lupus nephritis activity.[60] Khamchun et al.[61] demonstrated that the increased levels of serum ATT in SLE patients showed a strong correlation with CPR, complement C3 and C4, and ANA titer and could serve as a serum biomarker for the prognosis of disease severity. Together, these findings indicated that SERPINA1 might be a biomarker for monitoring the therapy response of MDA5$^+$DM patients, which is worth further exploration.

■ CONCLUSIONS

In summary, this work provided a valuable proteomics resource of plasma-derived exosomes from patients with DM and PM and identified possible biomarkers for clinical diagnosis. Our study showed that the DEPs of plasma-derived exosomes in both DM and PM patients were mainly enriched in the complement and coagulation cascade pathway,

suggesting that plasma-derived exosomes might participate in microvascular damage in DM/PM patients by activating the complement and coagulation cascade. In addition, our results demonstrated that the expression of SERPINA1 was significantly downregulated in post-treatment MDA5$^+$DM patients compared to pretreatment MDA5$^+$DM patients, implying its potential value for monitoring the therapy response. Thus, these findings provide valuable resources for the development of new diagnosis and therapeutic strategies for patients with DM or PM.

■ LIMITATIONS OF THE STUDY

Our study identified several potential biomarker candidates involved in disease pathogenesis and then validated them by PRM and ELISA. However, further studies in a larger cohort are needed in the future to identify these potential biomarkers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.2c00532.

All identified proteins by proteomics (Table S1) (XLSX)

Uncropped immunoblot membrane with molecular weight markers for exosome-specific markers (Figure S1); ROC curves for C1QC, FGA, FGB, and VWF showing the diagnostic capacity of DM and PM (Figure S2); detection of calnexin in the isolated particles by western blotting (Figure S3); and clinical characteristic and laboratory data of all patients for proteomics and ELISA (Table S2) (PDF)

Accession Codes

The proteomics and PRM data generated in this study have been uploaded to ProteomeXchange under accession nos. PXD037483 and PXD037608, respectively. All data are available for the corresponding author.

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Author Contributions

[§]S.M. and T.W. contributed equally to this study.

Author Contributions

X.P.H. and D.Z.L. designed and supervised the study; S.H.M. and T.T.W. performed experiments, drafted the manuscript, and analyzed the data; Q.Q.Z. helped revise the manuscript; Y.L.C. and Q.H. recruited patients; H.L. and C.L.L. collected samples; all authors reviewed the manuscript.

Notes

The authors declare no competing financial interest.

The present study was approved by the Ethics Committee of Shenzhen People's Hospital, China (LL-KY 2019514). All donors signed a written informed consent form to participate in this study.

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■ ABBREVIATIONS

IIMs, idiopathic inflammatory myopathies; DM, dermatomyositis; PM, polymyositis; HCs, healthy controls; LC−MS/MS, label-free liquid chromatography−mass spectrometry; PRM,

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parallel reaction monitoring; CRP, C-reaction protein; ESR, erythrocyte sedimentation rate; ROC curve, receiver operating characteristic curve; AUC, area under curve; MDA5, melanoma differentiation-associated protein 5; ILD, interstitial lung disease; 4D-LFQ, 4D label-free quantification; EULAR, European League Against Rheumatism; ACR, American College of Rheumatology; TEM, transmission electron microscopy; NTA, nanoparticle tracking analysis; DEPs, differentially expressed proteins; PDE, plasma-derived exosome; MAC, membrane attack complex

REFERENCES

(1) Dalakas, M. C. Inflammatory muscle diseases. *N. Engl. J. Med.* **2015**, *372*, 1734−1747.

(2) Gunawardena, H. The Clinical Features of Myositis-Associated Autoantibodies: a Review. *Clin. Rev. Allergy Immunol.* **2017**, *52*, 45−57.

(3) Iaccarino, L.; Ghirardello, A.; Bettio, S.; Zen, M.; Gatto, M.; Punzi, L.; Doria, A. The clinical features, diagnosis and classification of dermatomyositis. *J. Autoimmun.* **2014**, *48-49*, 122−127.

(4) Marie, I.; Hachulla, E.; Cherin, P.; Dominique, S.; Hatron, P. Y.; Hellot, M. F.; Devulder, B.; Herson, S.; Levesque, H.; Courtois, H. Interstitial lung disease in polymyositis and dermatomyositis. *Arthritis Rheum.* **2002**, *47*, 614−622.

(5) Sato, S.; Hirakata, M.; Kuwana, M.; Suwa, A.; Inada, S.; Mimori, T.; Nishikawa, T.; Oddis, C. V.; Ikeda, Y. Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. *Arthritis Rheum.* **2005**, *52*, 1571−1576.

(6) Fiorentino, D.; Chung, L.; Zwerner, J.; Rosen, A.; Casciola-Rosen, L. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. *J. Am. Acad. Dermatol.* **2011**, *65*, 25−34.

(7) Jani, M.; Massey, J.; Wedderburn, L. R.; Vencovsky, J.; Danko, K.; Lundberg, I. E.; Padyukov, L.; Selva-O'Callaghan, A.; Radstake, T.; Platt, H.; et al. Genotyping of immune-related genetic variants identifies TYK2 as a novel associated locus for idiopathic inflammatory myopathies. *Ann. Rheum. Dis.* **2014**, *73*, 1750−1752.

(8) Kochi, Y.; Kamatani, Y.; Kondo, Y.; Suzuki, A.; Kawakami, E.; Hiwa, R.; Momozawa, Y.; Fujimoto, M.; Jinnin, M.; Tanaka, Y.; et al. Splicing variant of WDFY4 augments MDA5 signalling and the risk of clinically amyopathic dermatomyositis. *Ann. Rheum. Dis.* **2018**, *77*, 602−611.

(9) Ye, L.; Zuo, Y.; Yang, H.; Li, W.; Peng, Q.; Lu, X.; Wang, G.; Shu, X. Specific Autoantibodies and Clinical Phenotypes Correlate with the Aberrant Expression of Immune-Related MicroRNAs in Dermatomyositis. *J. Immunol. Res.* **2019**, *2019*, No. 2927061.

(10) Moneta, G. M.; Pires Marafon, D.; Marasco, E.; Rosina, S.; Verardo, M.; Fiorillo, C.; Minetti, C.; Bracci-Laudiero, L.; Ravelli, A.; De Benedetti, F. Muscle Expression of Type I and Type II Interferons Is Increased in Juvenile Dermatomyositis and Related to Clinical and Histologic Features. *Arthritis Rheum.* **2019**, *71*, 1011−1021.

(11) Cappelletti, C.; Baggi, F.; Zolezzi, F.; Biancolini, D.; Beretta, O.; Severa, M.; Coccia, E. M.; Confalonieri, P.; Morandi, L.; Mora, M.; et al. Type I interferon and Toll-like receptor expression characterizes inflammatory myopathies. *Neurology* **2011**, *76*, 2079−2088.

(12) Bilgic, H.; Ytterberg, S. R.; Amin, S.; McNallan, K. T.; Wilson, J. C.; Koeuth, T.; Ellingson, S.; Newman, B.; Bauer, J. W.; Peterson, E. J.; et al. Interleukin-6 and type I interferon-regulated genes and chemokines mark disease activity in dermatomyositis. *Arthritis Rheum.* **2009**, *60*, 3436−3446.

(13) Suzuki, F.; Kubota, T.; Miyazaki, Y.; Ishikawa, K.; Ebisawa, M.; Hirohata, S.; Ogura, T.; Mizusawa, H.; Imai, T.; Miyasaka, N.; Nanki, T. Serum level of soluble CX3CL1/fractalkine is elevated in patients with polymyositis and dermatomyositis, which is correlated with disease activity. *Arthritis Res. Ther.* **2012**, *14*, No. R48.

(14) Kim, J.; Choi, J. Y.; Park, S. H.; Yang, S. H.; Park, J. A.; Shin, K.; Lee, E. Y.; Kawachi, H.; Kohsaka, H.; Song, Y. W. Therapeutic effect of anti-C-X-C motif chemokine 10 (CXCL10) antibody on C protein-induced myositis mouse. *Arthritis Res. Ther.* **2014**, *16*, R126.

(15) Xiao, Y.; Zhu, H.; Li, L.; Gao, S.; Liu, D.; Dai, B.; Li, Q.; Duan, H.; Yang, H.; Li, Q.; et al. Global analysis of protein expression in muscle tissues of dermatomyositis/polymyosisits patients demonstrated an association between dysferlin and human leucocyte antigen A. *Rheumatology* **2019**, *58*, 1474−1484.

(16) Li, S.; Yi, M.; Dong, B.; Jiao, Y.; Luo, S.; Wu, K. The roles of exosomes in cancer drug resistance and its therapeutic application. *Clin. Transl. Med.* **2020**, *10*, No. e257.

(17) Kalluri, R.; LeBleu, V. S. The biology, function, and biomedical applications of exosomes. *Science* **2020**, *367*, No. eaau6977.

(18) Tan, L.; Zhao, M.; Wu, H.; Zhang, Y.; Tong, X.; Gao, L.; Zhou, L.; Lu, Q.; Zeng, J. Downregulated Serum Exosomal miR-451a Expression Correlates With Renal Damage and Its Intercellular Communication Role in Systemic Lupus Erythematosus. *Front. Immunol.* **2021**, *12*, No. 630112.

(19) Perez-Hernandez, J.; Martinez-Arroyo, O.; Ortega, A.; Galera, M.; Solis-Salguero, M. A.; Chaves, F. J.; Redon, J.; Forner, M. J.; Cortes, R. Urinary exosomal miR-146a as a marker of albuminuria, activity changes and disease flares in lupus nephritis. *J. Nephrol.* **2021**, *34*, 1157−1167.

(20) Zhang, P.; Zhang, Y.; Pan, M.; Liu, Z.; Li, J.; Peng, L.; Zhou, J.; Hu, C.; Liu, S.; Zeng, X.; et al. Proteomic analyses of plasma-derived exosomes in immunoglobulin (Ig) G4-related disease and their potential roles in B cell differentiation and tissue damage. *J. Autoimmun.* **2021**, *122*, No. 102650.

(21) Lundberg, I. E.; Tjarnlund, A.; Bottai, M.; Werth, V. P.; Pilkington, C.; Visser, M.; Alfredsson, L.; Amato, A. A.; Barohn, R. J.; Liang, M. H.; et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann. Rheum. Dis.* **2017**, *76*, 1955−1964.

(22) Lou, Y.; Zheng, Y.; Fan, B.; Zhang, L.; Zhu, F.; Wang, X.; Chen, Z.; Tan, X.; Wei, Q. Serum levels of interleukins and S100A8/A9 correlate with clinical severity in patients with dermatomyositis-associated interstitial lung disease. *BMC Pulm. Med.* **2020**, *20*, No. 196.

(23) Bourmaud, A.; Gallien, S.; Domon, B. Parallel reaction monitoring using quadrupole-Orbitrap mass spectrometer: Principle and applications. *Proteomics* **2016**, *16*, 2146−2159.

(24) Momen-Heravi, F.; Getting, S. J.; Moschos, S. A. Extracellular vesicles and their nucleic acids for biomarker discovery. *Pharmacol. Ther.* **2018**, *192*, 170−187.

(25) Huang, T.; Deng, C. X. Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers. *Int. J. Biol. Sci.* **2019**, *15*, 1−11.

(26) Dunkelberger, J. R.; Song, W. C. Complement and its role in innate and adaptive immune responses. *Cell Res.* **2010**, *20*, 34−50.

(27) Leffler, J.; Bengtsson, A. A.; Blom, A. M. The complement system in systemic lupus erythematosus: an update. *Ann. Rheum. Dis.* **2014**, *73*, 1601−1606.

(28) Trivioli, G.; Vaglio, A. The rise of complement in ANCA-associated vasculitis: from marginal player to target of modern therapy. *Clin. Exp. Immunol.* **2020**, *202*, 403−406.

(29) Sturfelt, G.; Truedsson, L. Complement in the immunopathogenesis of rheumatic disease. *Nat. Rev. Rheumatol.* **2012**, *8*, 458−468.

(30) Lahoria, R.; Selcen, D.; Engel, A. G. Microvascular alterations and the role of complement in dermatomyositis. *Brain* **2016**, *139*, 1891−1903.

(31) Kissel, J. T.; Halterman, R. K.; Rammohan, K. W.; Mendell, J. R. The relationship of complement-mediated microvasculopathy to the histologic features and clinical duration of disease in dermatomyositis. *Arch. Neurol.* **1991**, *48*, 26−30.

(32) Jain, A.; Sharma, M. C.; Sarkar, C.; Bhatia, R.; Singh, S.; Gulati, S.; Handa, R. Detection of the membrane attack complex as a diagnostic tool in dermatomyositis. *Acta Neurol. Scand.* **2011**, *123*, 122−129.

(33) Li, L.; Chen, J.; Chen, S.; Liu, C.; Zhang, F.; Li, Y. Serum C1q Concentration Positively Correlates with Erythrocyte Sedimentation Rate in Polymyositis/Dermatomyositis. *Ann. Clin. Lab. Sci.* **2019**, *49*, 237−241.

(34) Challa, S.; Uppin, M. S.; Kannan, M. A.; Rajasekhar, L. C4d as a marker of complement activation in dermatomyositis muscle tissue. *Neurol. India* **2018**, *66*, 1062−1066.

(35) Shibabaw, T.; Molla, M. D.; Teferi, B.; Ayelign, B. Role of IFN and Complements System: Innate Immunity in SARS-CoV-2. *J. Inflammation Res.* **2020**, *13*, 507−518.

(36) Magro, C.; Mulvey, J. J.; Berlin, D.; Nuovo, G.; Salvatore, S.; Harp, J.; Baxter-Stoltzfus, A.; Laurence, J. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl. Res.* **2020**, *220*, 1−13.

(37) Morgan, B. P.; Harris, C. L. Complement, a target for therapy in inflammatory and degenerative diseases. *Nat. Rev. Drug Discovery* **2015**, *14*, 857−877.

(38) Wong, D.; Kea, B.; Pesich, R.; Higgs, B. W.; Zhu, W.; Brown, P.; Yao, Y.; Fiorentino, D. Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. *PLoS One* **2012**, *7*, No. e29161.

(39) Niewold, T. B.; Meves, A.; Lehman, J. S.; Popovic-Silwerfeldt, K.; Hayry, A.; Soderlund-Matell, T.; Charlesworth, C. M.; Madden, B.; Lundberg, I. E.; Wahren-Herlenius, M.; et al. Proteome study of cutaneous lupus erythematosus (CLE) and dermatomyositis skin lesions reveals IL-16 is differentially upregulated in CLE. *Arthritis Res. Ther.* **2021**, *23*, 132.

(40) Jodele, S.; Medvedovic, M.; Luebbering, N.; Chen, J.; Dandoy, C. E.; Laskin, B. L.; Davies, S. M. Interferon-complement loop in transplant-associated thrombotic microangiopathy. *Blood Adv.* **2020**, *4*, 1166−1177.

(41) Ryu, J. K.; Petersen, M. A.; Murray, S. G.; Baeten, K. M.; Meyer-Franke, A.; Chan, J. P.; Vagena, E.; Bedard, C.; Machado, M. R.; Rios Coronado, P. E.; et al. Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. *Nat. Commun.* **2015**, *6*, No. 8164.

(42) Willis, C. M.; Nicaise, A. M.; Menoret, A.; Ryu, J. K.; Mendiola, A. S.; Jellison, E. R.; Givogri, M. I.; Han, D. K.; Bongarzone, E. R.; Akassoglou, K.; et al. Extracellular vesicle fibrinogen induces encephalitogenic CD8+ T cells in a mouse model of multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 10488−10493.

(43) Denorme, F.; Vanhoorelbeke, K.; De Meyer, S. F. von Willebrand Factor and Platelet Glycoprotein Ib: A Thromboinflammatory Axis in Stroke. *Front. Immunol.* **2019**, *10*, No. 2884.

(44) Nombel, A.; Fabien, N.; Coutant, F. Dermatomyositis With Anti-MDA5 Antibodies: Bioclinical Features, Pathogenesis and Emerging Therapies. *Front. Immunol.* **2021**, *12*, No. 773352.

(45) Crowson, A. N.; Magro, C. M. The role of microvascular injury in the pathogenesis of cutaneous lesions of dermatomyositis. *Hum. Pathol.* **1996**, *27*, 15−19.

(46) Sun, Y. X.; Wright, H. T.; Janciauskiene, S. Glioma cell activation by Alzheimer's peptide Abeta1-42, alpha1-antichymotrypsin, and their mixture. *Cell. Mol. Life Sci.* **2002**, *59*, 1734−1743.

(47) Bodmer, J. L.; Schnebli, H. P.Plasma proteinase inhibitors *Schweiz. Med. Wochenschr.* 1984; Vol. *114* 40, pp 1359−1363.

(48) Baker, C.; Belbin, O.; Kalsheker, N.; Morgan, K. SERPINA3 (aka alpha-1-antichymotrypsin). *Front. Biosci.* **2007**, *12*, 2821−2835.

(49) Turnier, J. L.; Brunner, H. I.; Bennett, M.; Aleed, A.; Gulati, G.; Haffey, W. D.; Thornton, S.; Wagner, M.; Devarajan, P.; Witte, D.; et al. Discovery of SERPINA3 as a candidate urinary biomarker of lupus nephritis activity. *Rheumatology* **2019**, *58*, 321−330.

(50) Fissolo, N.; Matute-Blanch, C.; Osman, M.; Costa, C.; Pinteac, R.; Miro, B.; Sanchez, A.; Brito, V.; Dujmovic, I.; Voortman, M.; et al. CSF SERPINA3 Levels Are Elevated in Patients With Progressive MS. *Neurol Neuroimmunol. Neuroinflammation* **2021**, *8*, No. e941.

(51) Soric Hosman, I.; Kos, I.; Lamot, L. Serum Amyloid A in Inflammatory Rheumatic Diseases: A Compendious Review of a Renowned Biomarker. *Front. Immunol.* **2020**, *11*, No. 631299.

(52) Lee, J. Y.; Hall, J. A.; Kroehling, L.; Wu, L.; Najar, T.; Nguyen, H. H.; Lin, W. Y.; Yeung, S. T.; Silva, H. M.; Li, D.; et al. Serum Amyloid A Proteins Induce Pathogenic Th17 Cells and Promote Inflammatory Disease. *Cell* **2020**, *183*, 2036−2039.

(53) Ye, R. D.; Sun, L. Emerging functions of serum amyloid A in inflammation. *J. Leukocyte Biol.* **2015**, *98*, 923−929.

(54) Leung, C. L. L.; H, L.; Li, R. Z.; Pan, H. D.; Jiang, Z. B.; Li, Y.; Duan, F. G.; Xu, J. H.; Zhang, Y. Z.; Shi, A. X.; et al. Roles of Serum Amyloid A 1 Protein Isoforms in Rheumatoid Arthritis. *Engineering* **2022**, *10*, 174−182.

(55) Spicer, B. A.; Law, R. H. P.; Caradoc-Davies, T. T.; Ekkel, S. M.; Bayly-Jones, C.; Pang, S. S.; Conroy, P. J.; Ramm, G.; Radjainia, M.; Venugopal, H.; et al. The first transmembrane region of complement component-9 acts as a brake on its self-assembly. *Nat. Commun.* **2018**, *9*, No. 3266.

(56) Xu, W. D.; Liu, X. Y.; Su, L. C.; Huang, A. F. Association of MASP2 levels and MASP2 gene polymorphisms with systemic lupus erythematosus. *J. Cell. Mol. Med.* **2020**, *24*, 10432−10443.

(57) Janciauskiene, S. M.; Bals, R.; Koczulla, R.; Vogelmeier, C.; Köhnlein, T.; Welte, T. The discovery of alpha1-antitrypsin and its role in health and disease. *Respir. Med.* **2011**, *105*, 1129−1139.

(58) M Hunt, J.; Tuder, R. Alpha 1 anti-trypsin: one protein, many functions. *Curr. Mol. Med.* **2012**, *12*, 827−835.

(59) Stockley, R. A. The multiple facets of alpha-1-antitrypsin. *Ann. Transl. Med.* **2015**, *3*, No. 130.

(60) Aggarwal, A.; Gupta, R.; Negi, V. S.; Rajasekhar, L.; Misra, R.; Singh, P.; Chaturvedi, V.; Sinha, S. Urinary haptoglobin, alpha-1 anti-chymotrypsin and retinol binding protein identified by proteomics as potential biomarkers for lupus nephritis. *Clin. Exp. Immunol.* **2017**, *188*, 254−262.

(61) Khamchun, S.; Thakaeng, C.; Na Lampang, R. Serum alpha-1-antitrypsin level in the severity prognosis of systemic lupus erythematosus patients: Systematic exploration of novel biomarker. *Biomedicine* **2022**, *12*, 19−30.

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