CD44 is associated with muscle inflammation in polymyositis and skin damage in idiopathic inflammatory myopathy

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Abstract

Objective

Idiopathic inflammatory myopathy (IIM) is a group of systemic autoimmune diseases characterised by muscle involvement. This study aims to reveal the characteristics of IIM subtypes and explore the molecular mechanisms underlying IIM.

Methods

The STRING database was utilised to construct a protein-protein interaction network of differentially expressed genes obtained from the GSE128470, GSE3112, and GSE39454 datasets. The immune cell infiltration level was assessed by CIBERSORT in polymyositis (PM). Experimental autoimmune myositis (EAM) model mice were constructed for experimental verification. Serum levels of soluble CD44 (sCD44) were measured using enzyme-linked immunosorbent assay.

Results

The upregulated hub gene CD44 was highly expressed in inflammatory cells infiltrating the skeletal muscle of patients with PM and in EAM mice. CD44 was correlated with both M1 macrophages ($r=0.57$, $p<0.0001$) and M2 macrophages ($r=0.57$, $p<0.0001$) in PM. Additionally, $CD44^+F4/80^+$ macrophages in skeletal muscle were increased ($p<0.0001$) and CD44 showed a stronger association with markers of M1 macrophage in EAM mice. Moreover, serum sCD44 levels were elevated in patients with IIM ($p=0.0024$), PM ($p=0.0332$) and dermatomyositis ($p=0.0001$) notably in the anti-melanoma differentiation-associated gene 5 antibody positive subtype ($p=0.0007$). sCD44 levels also positively correlated with visual analogue score ($r=0.4424$, $p=0.0013$), myositis intention to treat activity index ($r=0.3938$, $p=0.0047$), skin damage score ($r=0.3796$, $p=0.0101$) and skin activity score ($r=0.4625$, $p=0.0014$) in patients with IIM.

Conclusion

This study suggests that macrophages expressing CD44 may be involved in the pathogenesis of PM, and sCD44 could serve as a potential marker for skin damage and activity in IIM.

Key words

CD44, experimental autoimmune myositis, idiopathic inflammatory myopathy, macrophages

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Introduction

Idiopathic inflammatory myopathy (IIM) is a group of systemic autoimmune connective tissue diseases, including dermatomyositis (DM), polymyositis (PM), immune-mediated necrotising myopathy (IMNM), antisynthetase syndrome (ASS), and other subtypes. IIM commonly presents with symmetrical proximal limb weakness and extra-muscular manifestations. Pathologically, IIM is characterised by immune cell infiltration into muscle fibres, accompanied by necrosis, regeneration, and degeneration of these fibres, along with inflammatory damage to skin and other tissues. The complex interplay of genetic and environmental factors may contribute to the occurrence and progression of the disease through immune or non-immune mechanisms (1, 2). Despite continued progress in research on IIM (3), the pathogenesis of IIM remains unclear. Revealing the characteristics of IIM and its subtypes will enhance our understanding of IIM and its pathogenesis.

Gene expression microarrays offer a novel approach to exploring genes, shedding light on the molecular mechanisms underlying IIM from various perspectives (4-8).

The cluster of differentiation 44 (CD44) acts as a receptor for hyaluronan and a coreceptor for receptor tyrosine kinases or G-protein-coupled receptors, playing a crucial role in inflammatory and immune diseases. CD44 has been implicated in the pathogenesis of rheumatoid arthritis (9) and diffuse scleroderma (10). A study found that in proteoglycan-induced arthritis, the expression of CD44 may facilitate the infiltration of innate immune cells into the joints (11). Another study demonstrated that targeting CD44 with a specific antibody could potentially treat osteoarthritis by suppressing macrophage activation (12).

Soluble CD44 (sCD44) is the soluble form of CD44 released by shear force. The levels and activity of sCD44 may vary under different pathophysiological conditions. Studies have indicated a potential association between sCD44 and the development, progression, and prognosis of diseases like cancer and autoimmune conditions such as rheumatoid arthritis (13-15).

Despite its known role in regulating inflammation and immunity, CD44 has not been extensively studied in IIM. Our study aims to explore the potential roles and mechanisms of CD44 and sCD44 in IIM.

Materials and methods

Microarray dataset information

We used the keyword "polymyositis" to search the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We further screened the project title, abstract, sample description and included the gene expression profiles of GSE128470, GSE3112, and GSE39454.

The detection platform for GSE128470 and GSE3112 (Affymetrix HG-U133A, Santa Clara, CA, USA) is GPL96, and the platform for GSE39454 (Affymetrix HG-U133 Plus 2.0 Array) is GPL570. From these three datasets, we selected a total of 49 individuals, including 28 controls and 21 patients with PM. The platform and series matrix files were downloaded from GEO.

The detection platform for GSE5370 (Affymetrix Human Genome U133A Array) is GPL96, and it contains gene expression profiles of affected skeletal muscle from normal controls (NCs) and patients with DM. The detection platform for GSE46239 (Affymetrix Human Gene U133 Plus 2.0 Array) is GPL570 and it contains gene expression profiles of skin biopsy specimens from NCs and patients with DM. We selected the dataset from the GPL14593 sequencing platform in the GSE32245 (Affymetrix Human Gene 1.0 ST Array), which contained gene expression profiles from skin biopsy specimens of NCs and DM patients with disease activity.

Microarray dataset processing and differentially expressed gene (DEG) identification

For the three datasets (GSE128470, GSE3112, and GSE39454), the probe matrices were converted into gene matrices using a Perl script (Perl 5, v. 26.3). Gene matrices were combined according to the gene symbols. The batch correction was performed using

Limma and SVA packages in R software (v. 4.1.0).

DEGs were screened for the combined gene matrix obtained from GSE5370, GSE46239 and GSE3224 using the Limma package with |log fold change (FC)| ≥1 and adjusted $p<0.05$. The smaller the adjusted p values, the greater the possibility of DEGs. The upregulated or downregulated DEGs were used for further analysis.

Protein-protein interaction (PPI) network construction and immune infiltration analysis

The STRING database (https://string-db.org, v. 11.5) was used to explore and analyse known and predicted PPIs. An integrated network was obtained after deleting all isolated nodes. The findings were used to identify the interactions and related pathways between proteins encoded by DEGs in PM.

We used CIBERSORT to analyse the combined and normalised gene matrices to obtain the immune cell infiltration matrix (22 types of immune cells were analysed). We used the psych package in R to perform a correlation analysis on the gene matrix of the hub genes and the immune cell matrix. Then we used the "ggcorrplot" package to visualise the data as a correlation matrix plot.

Construction of experimental autoimmune myositis (EAM) model mice

Eight-week-old female Balb/c mice (weighing approximately 18–20g), purchased from the HFKbio company (Beijing, China), were housed in the animal care facility of the West China Hospital. All protocols involving animals were reviewed and approved by the institutional animal welfare committee of West China Hospital, Sichuan University (no. 20220808002). EAM model mice were constructed as described previously (16). We randomly assigned mice to the control and EAM groups (6 mice per group). On the 1st and 7th days, potassium chloride solution containing 1.5 mg of myosin and an equal amount of Freund's complete adjuvant (Sigma-Aldrich) were emulsified, and the inactivated *Mycobacterium tuberculosis* (BD Difco) was added at 5 mg/ml and injected subcutaneously into the left hind limb of mice. Immediately after each immunisation, 200 μl of saline containing 500 ng of pertussis toxin (GLPbio) were injected intraperitoneally. Incomplete Freund's adjuvant (Sigma-Aldrich) emulsion containing myosin was injected subcutaneously at the base of the tail twice a week to boost the immune response. The control group was administered saline with complete Freund's adjuvant and pertussis toxin twice. We used 1% sodium pentobarbital to anesthetise the mice during the modelling procedure. The serum and left gastrocnemius muscle tissues of the mice were collected on day 14 after the first immunisation.

Clinical samples and data collection

Sixty-one peripheral blood samples (35 DM, 6 PM, 14 IMNM, 6 ASS) and 3 skeletal muscle specimens were collected from patients with PM at the Rheumatology and Immunology Department of West China Hospital, Sichuan University (6/2021–12/2022). All patients met the EULAR/ACR criteria (2017) for IIM (17). Patients diagnosed with IMNM also met the European Neuromuscular Centre (ENMC) criteria (2017) for IMNM (18). Patients diagnosed with ASS also met the diagnostic criteria proposed by Solomon *et al*. in 2011 (19). After excluding IMNM and ASS, the remaining patients who met the EULAR/ACR criteria (2017) for PM were diagnosed accordingly. Patients who were under 18 years of age, pregnant or breastfeeding women, individuals with other autoimmune diseases, tumours, HIV infection, chronic liver disease, or other muscle disorders, as well as patients who engaged in vigorous exercise within one week before recruitment, were excluded from the study. Simultaneously, peripheral blood samples were collected from twenty-nine age- and sex-matched healthy controls (HCs), and quadriceps muscle specimens (obtained during knee joint replacement surgery) were collected from three patients with osteoarthritis to serve as NCs. The hospital commissioned serum myositis-specific antibody testing, which was performed by Chengdu Huachuang Qide Medical Laboratory Limited Company using the immunoblotting method. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the West China Hospital, Sichuan University (no. 521, 2021). The Myositis Disease Activity Assessment Tool (MDAAT) and Myositis Intention to Treat Activity Index (MITAX) are used to assess disease activity and severity of IIM. The Cutaneous Assessment Tool (CAT) is used to assess skin and mucosal damage and activity of IIM. The Manual Muscle Testing 8 (MMT8) was used to assess muscle strength of IIM.

Real-time quantitative PCR

Total RNA was extracted from skeletal muscle tissue using Trizol (Ambion, USA) reagent according to the manufacturer's instructions. The cDNAs were synthesised from 1 μg of the total RNA in a 20 μl reaction system using 5X All-In-One RT Mastermix (abm, Zhenjing, China). Real-time quantitative PCR for individual target mRNA expression was performed with a CFX96™ Real-Time system (Bio-Rad, Hercules, CA, USA) using a ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China). The amount of specific mRNA in each sample was calculated based on the cycle threshold (CT) values, which were standardised with the housekeeping gene GAPDH. Further calculations and statistical analyses were performed using the comparative $2^{-\Delta\Delta CT}$ method. All the primer sequences are listed in Supplementary Table S1.

Western blot

Skeletal muscle tissues were homogenised in cold RIPA buffer (Beyotime, Jiangsu, China) containing a 1% proteinase inhibitor cocktail (Servicebio, Wuhan, China). Proteins (30 μg) were separated on a 10% SDS-PAGE gel and transferred to PVDF membranes (Millipore Corp, Bedford, MA, USA). Membranes were blocked in Tris-buffered saline containing 0.1% Tween-20 and 5% skim milk powder for one hour at room temperature. Next, the membranes were incubated with primary antibodies against GAPDH (1:10,000, Santa Cruz, Dallas, TX, USA) and CD44 (1:2000, Proteintech Group,

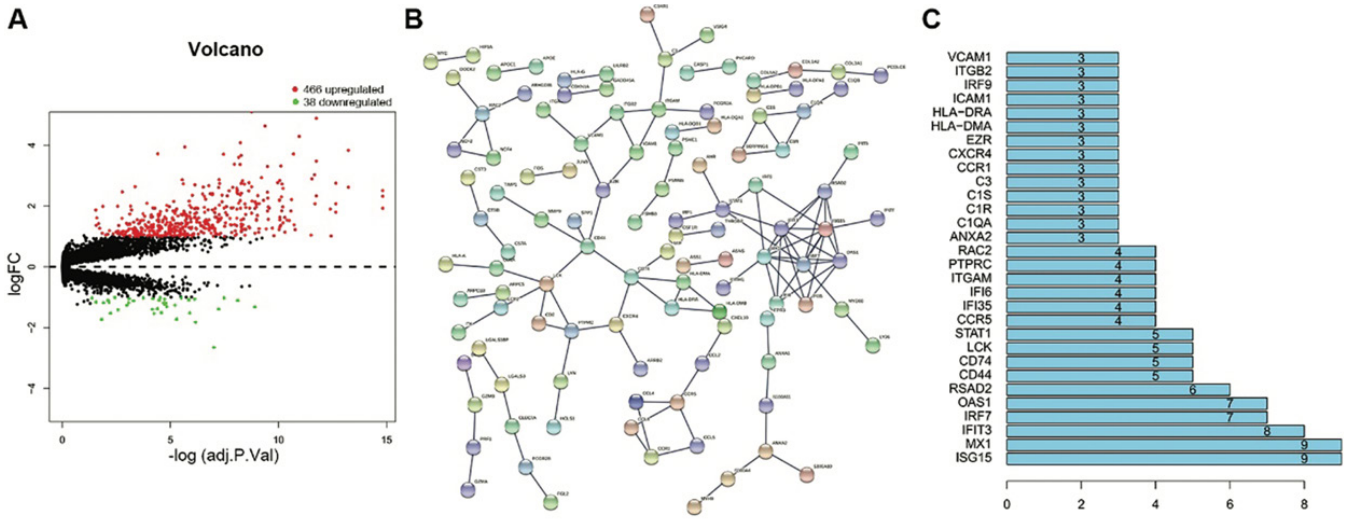

Fig. 1. The volcano plot and PPI network of DEGs. **A**: The volcano plot of the DEGs. Black points represent genes that are not significantly differentially expressed (|log FC| ≥1, adjusted p>0.05). The green points represent downregulated genes and the red points represent upregulated genes (|log FC| ≥1, adjusted p<0.05). **B**: The 504 DEGs were filtered into the PPI network that contains 111 nodes and 120 edges. **C**: The predicted association rank of the top 30 genes in the PPI network. Each network node represents a different protein, and the edges between these nodes represent the interrelationship between molecules. The X-axis represents the connectivity degree of the genes. DEGs: differentially expressed genes; PPI: protein-protein interactions.

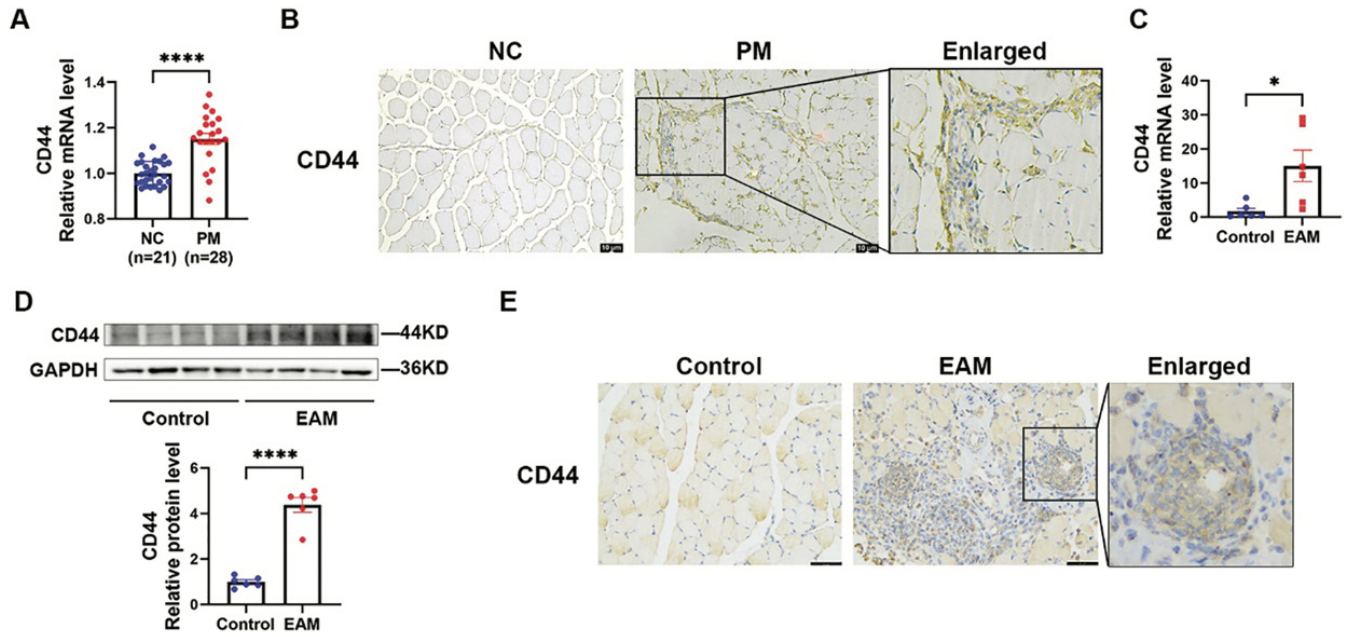

Fig. 2. CD44 is increased in the skeletal muscles of patients with PM and in EAM mice. **A**: mRNA levels of CD44 in skeletal muscle of NCs and patients with polymyositis. **B**: Immunohistochemical staining of CD44 in the muscles of NCs and patients with PM (n=3). Scale bar = 10 μm. **C**: mRNA levels of CD44 in the muscles of mice (n=6). **D**: Western blot and strip analyses of CD44 in the muscles of mice (n=6). **E**: Immunohistochemical staining of CD44 in the muscles of mice (n=6). Scale bar = 50 μm.
NC: normal control; PM: polymyositis; EAM: experimental autoimmune myositis. Data were expressed as mean ± SEM; *p<0.05, ****p<0.0001.

Wuhan, China) at 4°C overnight, followed by incubation with a secondary HRP-conjugated IgG (1:1000, Proteintech Group) for one hour at room temperature. Immunoreactive proteins were visualised using a chemiluminescence imaging system (Bio-Rad, California, USA). Protein banding analysis was performed using ImageJ.

Immunostaining

Skeletal muscle tissues were embedded in wax blocks and sectioned. After deparaffinisation, the tissues were subjected to antigen retrieval with citric acid buffer. Tissues were blocked with 3% hydrogen peroxide solution and goat-derived serum, and the CD44 (1:300, Proteintech Group, Wuhan, China) antibody was added and incubated at 4°C overnight. The tissue was covered with a rabbit secondary antibody (HRP labelled) and incubated at room temperature for 50 min. Subsequently, DAB chromogenic reaction (Zsbio, Beijing, China) and haematoxylin counterstaining were performed. The tissues were observed under a mi-

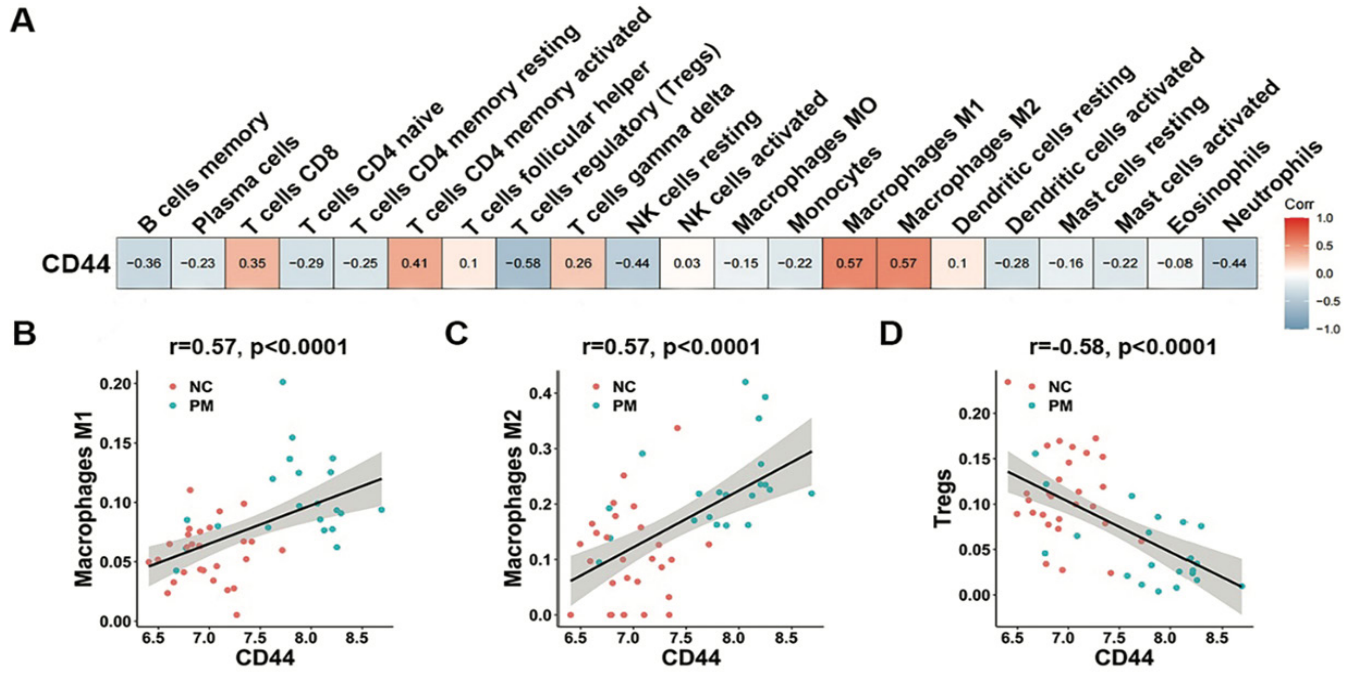

Fig. 3. CD44 is positively correlated with macrophages and negatively correlated with Tregs in NC and PM.
A: Correlation matrix between CD44 and infiltrating immune cells. Red dots indicate positive correlation trends, and blue dots indicate negative correlation trends between hub genes and immune cells. **B**: Correlation between CD44 and M1 macrophages or M2 macrophages (**C**) or Tregs (**D**) in the muscles of NC and PM.
NC: normal control; PM: polymyositis; Tregs: regulatory T cells. ****p<0.0001.

croscope (Leica, Wetzlar, Germany), and the brownish-yellow-stained regions showed antibody localisation.

Frozen sections were fixed and incubated overnight at 4°C with antibodies against F4/80 (Abcam, USA) at 1:100 dilution and CD44 (Proteintech Group, Wuhan, China) at 1:300 dilution. Following a one hour incubation in the dark with CY3- or FITC-conjugated secondary antibodies (1:200, Abcam, USA), the nucleus was stained with DAPI (Servicebio, Wuhan, China) for 5 minutes before microscopic analysis (Leica DM4 B, Germany). As a negative control, the primary antibodies were replaced by preimmune IgG from the same species; little or no non-specific staining occurred. Images of skeletal muscle tissues were randomly taken (original magnification × 400, 5 fields of view per slice) from each mouse (n=6). The number of $CD44^+$/$F4/80^+$ and $F4/80^+$ cells was quantified using ImageJ and the percentage of $CD44^+/F4/80^+$ among total $F4/80^+$ cells was calculated.

Enzyme-linked immunosorbent assay

The serum sCD44 levels were detected by an enzyme-linked immunosorbent assay kit (Ruixinbio, Quanzhou, China). Following the provided instructions, the process involved sample addition, antibody treatment, incubation, colour development, and termination. After the stop solution was added, and within 15 minutes, the absorbance (OD values) of each well was read using an enzyme-linked immunosorbent assay analyser. Four-parameter Logistic curve fitting was used to create a standard curve equation, which was used to calculate the sample concentration using the OD values.

Statistical analysis

Data were expressed as mean ± SEM. SPSS 20 (IBM, NY, USA) and GraphPad Prism 9 (CA, USA) were used to perform statistical analyses and to build the graphs presented in this paper, respectively. Statistical comparisons between two groups were determined by two-tailed Student's *t*-test or Mann-Whitney U-test. One-way ANOVA with Tukey's or Dunnett's *post-hoc* test was performed for comparisons among multiple groups. Pearson correlation analysis was used for variables with a normal distribution, and Spearman correlation analysis was used for variables with a non-normal distribution. The p-value <0.05 was considered statistically significant.

Results

PPI network construction and hub gene analysis

A total of 504 DEGs were identified, with 466 upregulated and 38 downregulated in the volcano map (Fig. 1A). The PPI network of DEGs in patients with PM was constructed using a minimum interaction score of 0.990, as shown in Figure 3B. Figure 3C shows the top 30 hub genes with statistically significant interactions. Among these genes, interferon-stimulated gene 15 (ISG15), myxovirus resistance 1 (MX1), interferon-induced protein with tetratricopeptide repeats 3 (IFIT3), interferon regulatory factor 7 (IRF7), oligoadenylate synthetase 1 (OAS1), radical s-adenosyl methionine domain containing 2 (RSAD2), CD44, CD74, lymphocyte-specific protein tyrosine kinase (LCK), and signal transducer and activator of transcription 1 (STAT1) had the highest node degrees. These hub genes can be

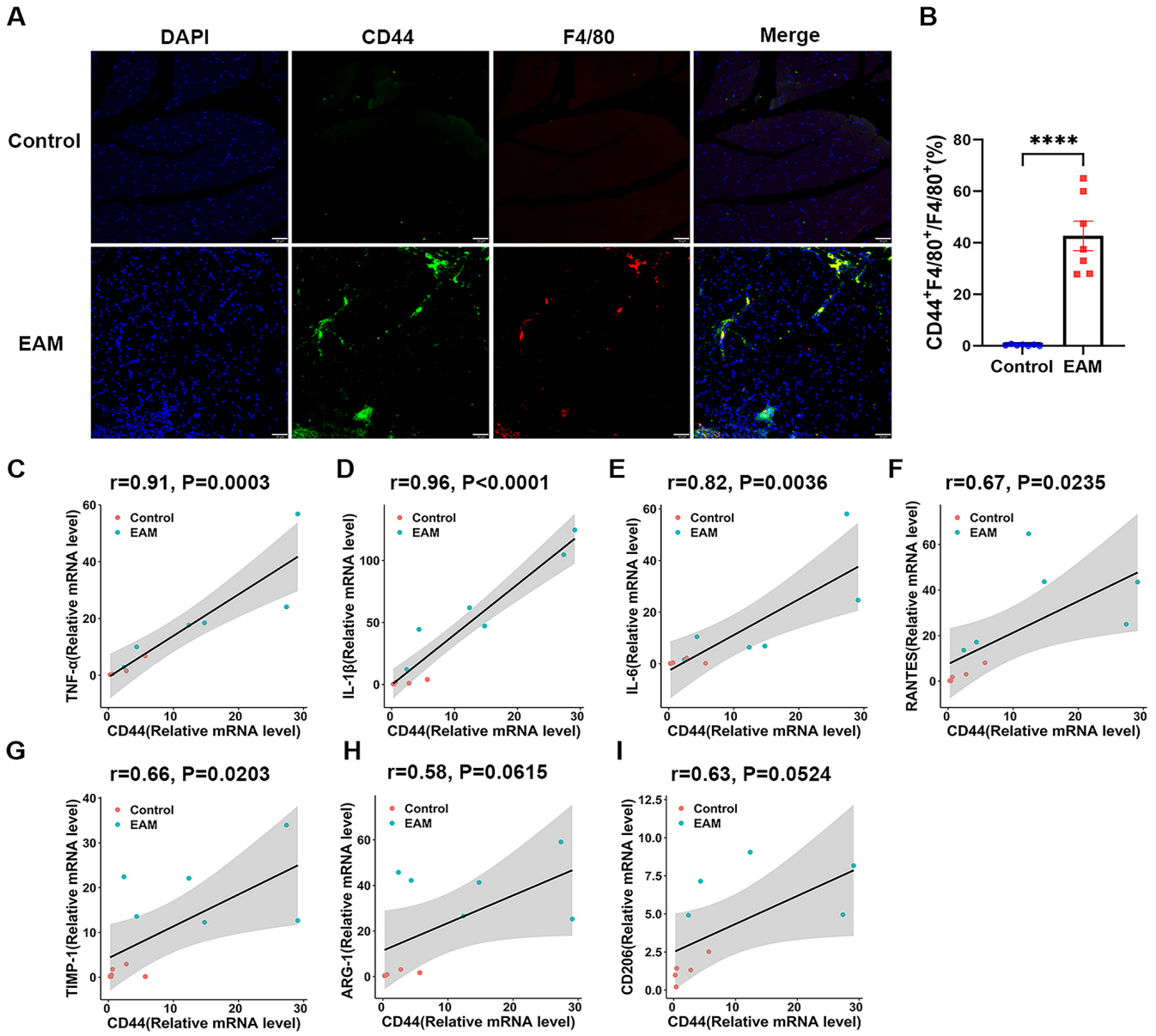

Fig. 4. CD44 is associated with macrophage polarisation in skeletal muscles of patients with PM and in EAM mice.
A: Immunofluorescence staining of CD44$^+$ (green)/F4/80$^+$ (red) cells in the skeletal muscles of mice. Nuclei were counterstained with DAPI (blue). Scale bar=50 μm.
B: The proportion of CD44$^+$/F4/80$^+$ cells in total F4/80$^+$ cells (n=7). Correlation of mRNA levels between CD44 and macrophage polarisation markers of TNF-α (**C**), IL-1β (**D**), IL-6 (**E**), RANTES (**F**), TIMP1 (**G**), ARG-1 (**H**) and CD206 (**I**) (n=10-12).
TNF-α: tumour necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-6: interleukin-6; RANTES: the regulated upon activation normal T cell expressed and secreted factor; TIMP1: tissue inhibitor of metalloproteinases 1; Arg-1: arginase-1.
Data were expressed as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

broadly categorised into two groups: an interactive network comprising interferon (IFN)-related genes and another network centred on a loop consisting of CD74, CD44, LCK, PTPRC, and CXCR4.

CD44 is increased in skeletal muscles of patients with PM and in EAM mice

The above hub genes, such as IFN-related genes, CD74, and STAT1 have been studied in IIM (20-30). However, there is limited research on the role of CD44 in IIM. Our analysis of multiple IIM transcriptome datasets revealed elevated CD44 levels in PM compared to NCs, but not in DM (Suppl. Fig. S1). Similarly, there was a significant increase in CD44 expression in integrated PM dataset (GSE128470, GSE3112, GSE39454) compared to NCs (p<0.0001) (Fig. 2A). Notably, both the gene (p<0.05) (Fig. 2C) and protein (p<0.0001) (Fig. 2D) levels of CD44 were significantly higher in the skeletal muscle of EAM mice compared to the controls. Immunohistochemical staining demonstrated that CD44 was predominantly expressed in immune cells infiltrating the skeletal muscle of both patient with PM (Fig. 2B) and EAM mice (Fig. 2E).

Table I. Clinical data of HCs and patients with IIM.

Items	Patients with IIM	Healthy	*p*-value
Females	44/61 (69%)	25/29 (86%)	0.1858
Age (years)	49 (43, 55)	47 (43, 53)	0.5249
Subtypes of IIM			
DM	35 (57%)		
PM	6 (10%)		
IMNM	14 (23%)		
ASS	6 (10%)		
Myositis-specific antibodies			
Anti-MDA5	22 (36%)		
Anti-TIF1-γ	1 (2%)		
Anti-Mi-2β	1 (2%)		
Anti-SRP	8 (13%)		
Anti-HMGCR	4 (7%)		
Anti-ARS	11 (18%)		
MSA-negative	8 (13%)		
Biochemical parameters			
Creatinine kinase (IU/L)	150 (48, 2156)		
CRP (mg/L)	4.61 (2.10, 11.68)		
ESR (mm/h)	44.00 (22.50, 62.50)		
C3 (g/L)	0.912 (0.776, 1.020)		
C4 (g/L)	0.264 (0.188, 0.322)		
Myositis activity score			
MYOACT score of extra-muscular organ	2.00 (1.29, 3.17)		
VAS score of muscle	4.00 (1.75, 6.00)		
VAS score of skin	3 (2, 5)		
MITAX score of extra-muscular organs	1.50 (0.67, 2.50)		
MITAX score of muscle	1 (1, 3)		
MITAX score of skin	3 (1, 3)		
CAT score of skin activity	3 (1, 6)		
CAT score of skin damage	3 (2, 4)		
MMT8	138 (105, 150)		

Data is represented as n (%) or median (25th percentile, 75th percentile).
IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; PM: polymyositis; IMNM: immune-mediated necrotising myopathy; ASS: antisynthetase syndrome; MDA5: melanoma differentiation-associated gene 5; SRP: signal recognition particle; HMGCR: hydroxymethylglutaryl CoA reductase; TIF1-γ: transcriptional intermediary factor 1-γ. ARS: aminoacyl-transfer ribonucleic acid synthetase; MSA: myositis-specific autoantibody; CK: creatine kinase; MYOACT: myositis disease activity assessment visual analogue scale; VAS: visual analogue score; MITAX: myositis intention to treat activity index; CAT: cutaneous assessment tool; MMT8: manual muscle testing 8. *$p<0.05$.

CD44 is associated with macrophage polarisation in skeletal muscles of patients with PM and in EAM mice

CD44 shows positive correlations with M1 macrophages (r=0.57, $p<0.0001$) (Fig. 3A and B) and M2 macrophages (r=0.57, $p<0.0001$) (Fig. 3A and C) in NC and PM, while exhibiting a negative correlation with regulatory T cells (Tregs) (r=-0.58, $p<0.0001$) (Fig. 3A and D). Immunofluorescence staining illustrated the co-localisation of CD44 and F4/80 (Fig. 4A), with an increase in $CD44^+F4/80^+$ macrophages ($p<0.0001$) (Fig. 4B) in the skeletal muscle of EAM mice. Moreover, at the mRNA levels, CD44 exhibited a strong correlation with M1 macrophage markers, including tumour necrosis factor-α (TNF-α) (r=0.91, $p=0.0003$) (Fig. 4C), interleukin (IL)-1β (r=0.96, $p<0.0001$) (Fig. 4D), IL-6 (r=0.82, $p=0.0036$) (Fig. 4E), and the regulated upon activation normal T cell expressed and secreted factor (RANTES) (r=0.67, $p=0.0235$) (Fig. 4F). CD44 showed a relatively weaker correlation with M2 macrophage marker tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) (r=0.66, $p=0.0203$) (Fig. 4G), and no significant correlations with arginase (ARG)-1 (r=0.58, $p=0.0615$) (Fig. 4H) and CD206 (r=0.63, $p=0.0524$) (Fig. 4I).

Serum sCD44 levels are increased in patients with DM and PM

The clinical data of HCs and patients with IIM included in our study are shown in Table I. Patients with IIM exhibited elevated serum levels of sCD44 (532.8 *vs*. 438.4 pg/mL; $p=0.0024$) (Fig. 5A) compared to HCs. Figure 5B shows that upon further subclassification of IIM, patients with DM (581.5 pg/mL; $p=0.0001$) and PM (599.2 pg/mL; $p=0.0332$) had elevated serum sCD44 levels compared to HCs, while patients with ASS exhibited lower serum sCD44 levels (400.7 pg/mL; *vs*. DM, $p=0.0076$; *vs*. PM, $p=0.0371$) compared to patients with DM and PM. Furthermore, upon classification based on myositis-specific antibodies (MSA), DM patients with positive anti-melanoma differentiation-associated gene 5 ($anti\text{-}MDA5^+$) antibody exhibited significantly higher serum sCD44 levels (591.8 pg/mL; $p=0.0007$) (Fig. 5C) compared to HCs.

sCD44 is positively correlated with skin damage and activity in patients with IIM

Correlational analysis between serum sCD44 levels and clinical indicators revealed a positive correlation between serum sCD44 levels and the VAS scores of skin (r=0.4424, $p=0.0013$) (Fig. 6A), MITAX scores of skin (r=0.3938, $p=0.0047$) (Fig. 6B), skin damage scores (r=0.3796, $p=0.0101$) (Fig. 6C), and skin activity scores (r=0.4625, $p=0.0014$) (Fig. 6D) in IIM. Moreover, analysis of the public dataset GSE46239 showed a trend towards elevated mRNA levels of CD44 in the skin of patients with DM compared to NCs, although this difference did not reach statistical significance ($p=0.0587$) (Fig. 6E). In GSE32245, mRNA levels of CD44 were significantly elevated in the skin of DM patients with high disease activity compared to the controls ($p=0.0163$) (Fig. 6F).

Discussion

Given the unclear pathogenesis of IIM, it is imperative to thoroughly investigate the characteristics and potential molecular mechanisms of IIM subtypes. Although the three datasets we selected have been used for bioinformatics analysis in previous research (6, 31-34), there remains significant potential for exploring the pathogenesis of IIM using the wealth of information generated from bioinformatics analysis of sequencing data.

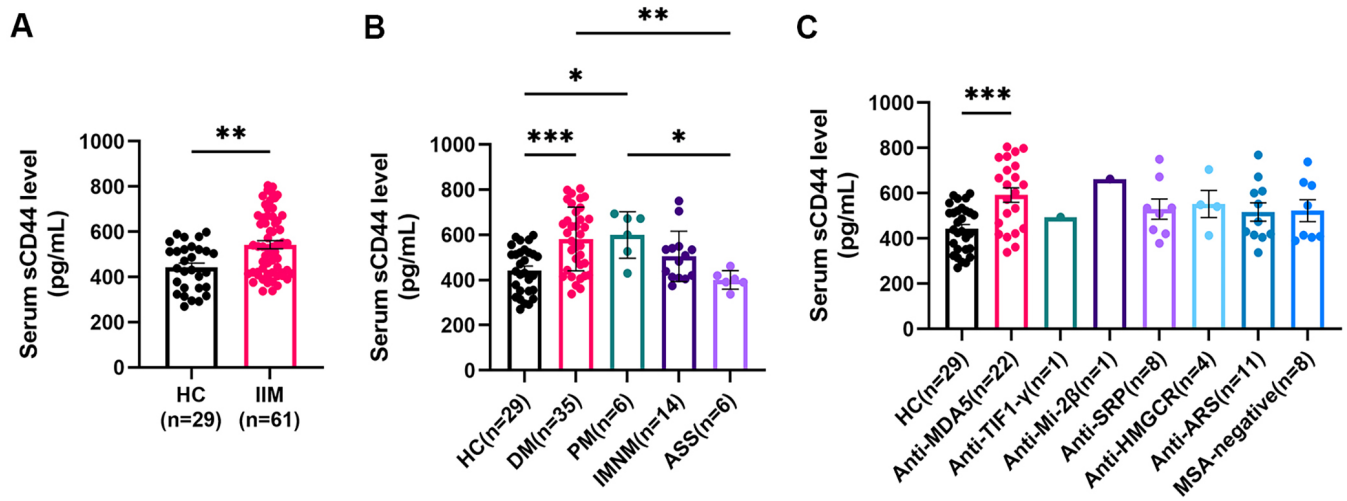

Fig. 5. Serum sCD44 levels are elevated in patients with DM and PM.
A: Serum sCD44 levels were elevated in patients with IIM compared to HC. **B**: Serum sCD44 levels were elevated in patients with DM and PM. **C**: Serum sCD44 levels were elevated in DM patients who were positive for anti-MDA5 antibody.
sCD44: soluble CD44; IIM: idiopathic inflammatory myopathy; HC: healthy control; DM: dermatomyositis; PM: polymyositis; IMNM: immune-mediated necrotizing myopathy; ASS: antisynthetase syndrome; MDA5: melanoma differentiation-associated gene 5; SRP: signal recognition particle; HMGCR: hydroxymethylglutaryl CoA reductase; TIF1-γ: transcriptional intermediary factor 1-γ; ARS: aminoacyl-transfer ribonucleic acid synthetase; MSA: myositis-specific autoantibody.
Data were expressed as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001.

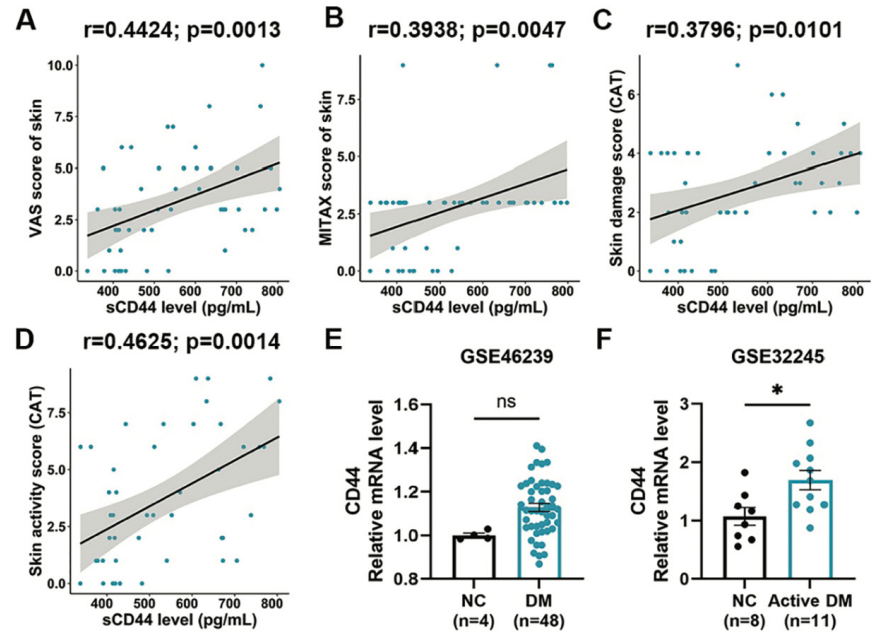

Fig. 6. Serum sCD44 levels are positively correlated with skin damage and activity in patients with IIM. Correlation plots of serum sCD44 levels with skin scores of VAS (**A**), skin scores of MITAX (**B**), skin damage scores of CAT (**C**), and skin activity scores of CAT (**D**). n=45-50. The CD44 mRNA levels were analysed in NC and DM in the transcriptome datasets of GSE46239 (**E**) and GSE32245 (**F**).
sCD44: soluble CD44; NC: normal control; IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; VAS: visual analogue score; MITAX: myositis intention-to-treat activity index; CAT: cutaneous assessment tool.
Data were expressed as mean ± SEM; *p<0.05.

We constructed a PPI network of proteins encoded by DEGs. This network can be roughly divided into two main parts: one part consists of an interaction network formed by IFN-related genes, highlighting the crucial role of the IFN-related signalling pathway in the pathogenesis of IIM (35, 36); the other part is a cyclic network comprising CD74, CD44, LCK, PTPRC, and CXCR4. We found that among the top 10 hub genes, ISG15, MX1, IFIT3, IRF7, OAS1, RSAD2, CD74, and STAT1 have been implicated in previous research related to IIM (20-22, 26, 29, 30, 34, 37-40), except for CD44 and LCK. Studies have found that functional activation of CD44 in immune cells may be a potential indicator of autoimmune disease activity (41). Moreover, CD44 or macrophages expressing CD44 have been reported to play a significant role in inflammatory diseases and are linked to macrophage polarisation (9, 42-44). Consistent with this, our study found that CD44 was positively correlated with both M1 and M2 macrophages in PM, while CD44-expressing macrophages in the affected muscles of EAM mice were more closely associated with M1 macrophage polarisation, which led us to speculate that the regulatory role of CD44 on macrophages depends on the stage of the disease. Recently, numerous studies have been conducted on regulating macrophage polarisation by drug-loaded hyaluronic acid nanoparticles, targeting CD44, potentially leading to disease regression (45, 46), which suggests that targeting CD44 could be beneficial for treating PM by regulating macrophage polarisation. However, further study is neces-

sary to clarify the importance of CD44 in PM and the intrinsic mechanisms of its effects on macrophage polarisation. In addition, CD44 was found to mediate signal transduction involving LCK (47, 48). As a non-receptor tyrosine kinase, LCK is known to regulate TCR signal transduction, T-cell development, and T-cell homeostasis (49). Therefore, exploring the role of LCK in PM in our future research is warranted.

To identify the distinctive molecular signature of PM using the simplest possible assay, we measured serum sCD44 levels in patients with IIM. Our results showed elevated sCD44 levels in both DM and PM, with the highest levels observed in the anti-MDA5 antibody-positive DM subtype. Previous studies have long found elevated serum soluble CD44 variant isoforms in rheumatoid arthritis, although there is controversy over whether these isoforms can reflect the clinical course of the disease (15, 50). We found that sCD44 might be related to skin damage and activity in patients with IIM. Moreover, the mRNA expression level of CD44 was found to be higher in the affected skin of patients with active DM compared to the NCs in the GSE32245 dataset. Previous studies have shown that CD44 expression levels increased in UV irradiation-induced precancerous hyperplasia (51) and in psoriasis lesions (52), and CD44 is closely associated with the wound healing process (53) and melanoma metastasis (54). Notably, a study found that the accumulation of CD44v7 is a unique molecular feature observed in Gottron's papules of patients with DM (55). Our results, in conjunction with existing literature, suggest that CD44 and sCD44 may be involved in the pathological skin damage mechanisms of patients with IIM. Nevertheless, the specific functions and mechanisms of different CD44 shear isoforms in skin damage across various subtypes of IIM patients require further investigation.

In conclusion, this study highlights the potential role of CD44-expressing macrophages in the development of PM and suggests that serum sCD44 could serve as a promising marker for assessing skin damage and activity in patients with IIM.

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GEO belongs to the public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles.

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