MDA5+ Dermatomyositis Is Associated with Stronger Skin Type I Interferon Transcriptomic Signature with Upregulation of IFN-κ Transcript

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MDA5$^+$ Dermatomyositis Is Associated with Stronger Skin Type I Interferon Transcriptomic Signature with Upregulation of IFN-κ Transcript

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TO THE EDITOR

Dermatomyositis (DM) is a chronic inflammatory and autoimmune disorder belonging to the idiopathic inflammatory myopathies affecting primarily skin and muscle with variable extent. Disease mechanism comprehension is growing, with a pivotal role of both the innate and adaptive immune system and involvement of both cellular and humoral actors (Nagaraju and Lundberg, 2011). The immune transcriptomic signature of DM is characterized by a type I IFN signature in peripheral blood mononuclear cells, muscle, and skin, which has been shown to correlate with disease activity (Baechler et al., 2011; Walsh et al., 2007; Wong et al., 2012); consequently, targeting type I IFN seems to represent a novel therapeutic approach in DM (Higgs et al., 2014). Among autoantibodies, MDA5 antibodies are associated with a unique and severe mucocutaneous presentation and a peculiar systemic phenotype with lower incidence of myositis, a higher ferritin level, an elevated risk of interstitial lung disease, and higher mortality (Kurtzman and Vleugels, 2018). Herein, we assessed the global gene expression, and more peculiarly the type I IFN signature, in the skin of three individuals with MDA5$^+$ DM and compared it with five individuals with MDA5$^-$ DM and five healthy individuals (Supplementary Table S1). (Supplementary Materials and Methods). The study has been approved by institutional review boards and was conducted in accordance with the ethical and legal frameworks of the Declaration of Helsinki. Informed written consent was received from participants before inclusion in this study, according to our local ethic rules (CPP Paris 12).

First, principal component analysis (unsupervised analysis) clustering using all 24,063 genes segregated healthy patients and patients with DM (Figure 1a and b) (Supplementary Figure S1). Using significance analysis of microarray (Tusher et al., 2001), we identified 575 differentially expressed genes in DM compared with healthy skin. As expected and previously described (Wong et al., 2012), upregulated genes and enrichment analysis pathway supported increased cellular infiltration of T cells (*CD3D*) and cytotoxic cells (*GZMA* and *GZMH*); activation of immune cells (*HLA-DR*); and upregulation of type I IFN pathway, RIG-I, IL2-STAT5, complement pathway, and natural killer cytotoxicity pathway in DM compared with healthy skin (Table 1). Gene expression of immune biomarkers previously described in the serum (Cassius et al., 2019) were also upregulated in the skin (*IL15* [fold change (FC) = 2.27; q = 0.002], *CXCL10* [FC = 12.55; q = 0.004], and *CD163* [FC = 4.2; q = 0.002]), confirming their potential pathogenic role. Concerning skin physiology, apoptosis and epithelial mesenchymal transition pathways were upregulated, supporting the cytotoxicity of inflammatory infiltrate toward keratinocytes (Table 1).

Hierarchical clustering segregated MDA5$^+$ from MDA5$^-$ DM, highlighting pathogeny differences and potential mechanisms of the distinctive clinical skin findings in this subgroup (Figure 1b). We therefore compared differentially expressed genes and pathways across MDA5$^+$ versus MDA5$^-$ DM to identify contrasting elements. Considering genes that had a minimum FC of 2, the principal component analysis clustered independently according to the serology, indicating distinct molecular profiles (Figure 1a). By comparing MDA5$^+$ versus normal skin and MDA5$^-$ versus normal skin, we found 305 genes differentially regulated in MDA5$^+$ skin and 100 genes differentially regulated in MDA5$^-$ skin (Figure 1c). Among the most highly upregulated genes in MDA5$^+$ skin were *IFIT2*, *CXCL10*, and *IFIH1*; the type I IFN pathway was also significantly upregulated (Table 1). To confirm the type I IFN signature in MDA5$^+$ DM, we showed using MxA immunohistochemistry that MDA5$^+$ DM skin samples expressed more MxA in the epidermis and in the inflammatory infiltrate than MDA5$^-$ skin samples (Figure 1d) (Supplementary Table S2). *E-selectin* gene expression was significantly upregulated in MDA5$^+$ skin compared with MDA5$^-$ skin (FC = 11.3, q = 0.02), highlighting the intense vasculitis phenomenon, clinically and histologically observed in practice.

Finally, to decipher the origin of the type I IFN signature in DM skin, we focused on both the cell origin of interferons and the type of interferons. Type I IFN signature can originate either from type I IFN (α, β, κ, ε, or ω), among which IFN-α is more specifically secreted by plasmacytoid dendritic cells and IFN-κ is more specifically secreted by keratinocytes, or from type III IFN (λ) (Li et al., 2018). The *IFNK* gene was the only interferon significantly elevated in MDA5$^+$ skin (FC = 2.27, q = 0.03) compared with healthy skin (Figure 1e). In MDA5$^-$ compared with healthy skin, *IFNK* was elevated but not significantly (FC = 1.9, q = 0.25). Plasmacytoid dendritic cells express CD123 (or IL3Rα); *CD123* gene expression was significantly elevated in DM samples and we observed the same tendency at the protein level by

Abbreviations: DM, dermatomyositis; FC, fold change

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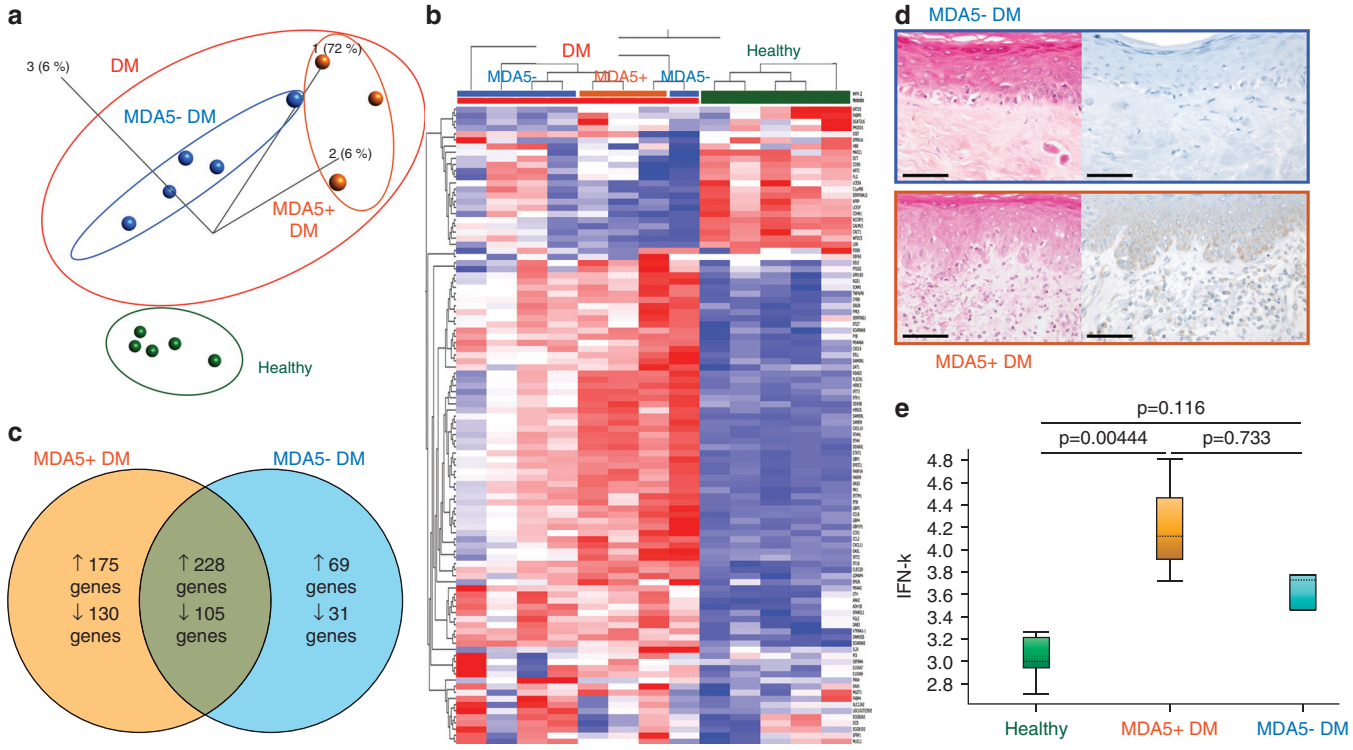

Figure 1. Transcriptomic profiling of skin distinguishes MDA5$^+$ from MDA5$^-$ DM. (**a**) Principal component analysis of microarray expression data of 13 skin samples showing the distance between MDA5$^+$, MDA5$^-$, and healthy patients. (**b**) Two-dimensional hierarchical clustering was performed on a set of 104 genes whose average expression significantly differed between MDA5$^+$, MDA5$^-$, and HS. All values are in $\log_2$ space and are mean-centered across each gene. (**c**) Venn diagram comparing DEGs with more than two-fold change between MDA5$^+$ (orange) and MDA5$^-$ (blue) compared with HS. (**d**) Histological section of MDA5$^-$ and MDA5$^+$ DM skin samples. HES (left) and MxA (right) immunohistochemistry. ×400 magnification, Bar = 100 μm. (**e**) Box and whisker plot representation of the expression of *IFNK* gene in MDA5$^+$ (n = 3) (orange) compared with MDA5$^-$ (n = 5) (blue) and HS (n = 5) (green). Microarray expression data in $\log_2$ scale. DEG, differentially expressed gene; DM, dermatomyositis; HS, healthy skin.

immunohistochemistry (Supplementary Table S2).

To confirm the mRNA overexpression of the type I IFN signature and *IFNK* genes in patients with MDA5$^+$ compared with patients with MDA5$^-$, we analyzed 19 more formalin-fixed paraffin-embedded skin samples from a validation cohort (10 patients with MDA5$^+$ and nine patients with MDA5$^-$) using reverse transcriptase–PCR (Supplementary Materials and Methods). *IFNK* expression was significantly higher in the skin of patients with MDA5$^+$ than patients with MDA5$^-$ (median: 57.6 vs. 0.7, $P = 0.03$); type I IFN signature was also higher in patients with MDA5$^+$ than patients with MDA5$^-$ DM (median: 556 vs. 87, $P = 0.02$) (Supplementary Figure S2).

We confirm here that DM skin involvement is associated with a specific transcriptomic signature comprising activation and infiltration of immune cells and a type I IFN signature. Previous work showed that, when studied in the muscle, IFN

Table 1. GSEA Analysis of Upregulated and Downregulated Genes in DM

Name	Size	ES	NES	*P*-value	FDR
GSEA analysis of upregulated genes in DM compared with healthy controls					
H IFN-α response	80	0.86	2.97	0.000	0.000
H complement	173	0.65	2.50	0.000	0.000
KEGG natural killer cell–mediated cytotoxicity	119	0.50	1.83	0.000	0.005
H inflammatory response	169	0.63	2.43	0.000	0.000
H epithelial mesenchymal transition	165	0.64	2.51	0.000	0.000
H apoptosis	133	0.47	1.75	0.000	0.002
GSEA analysis of upregulated genes in MDA5$^+$ compared with MDA5$^-$ DM					
H IFN-α response	80	0.74	2.71	0.000	0.000
H IFN-γ response	168	0.65	2.63	0.000	0.000
H TNF-α signaling via NF-κB	172	0.62	2.52	0.000	0.000
H inflammatory response	169	0.57	2.34	0.000	0.000

Abbreviations: DM, dermatomyositis; ES, enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; H, hallmark; KEGG, Kyoto Encyclopedia of Genes and Genomes; NES, normalized enrichment score; TNF-α, tumor necrosis factor-α.

signature was higher in MDA5− compared with MDA5+ DM (Allenbach et al., 2016). Our data suggests that the type I IFN signature is expressed more strongly in the skin of MDA5+ than MDA5− DM. This discrepancy could be explained by the organ analyzed, as skin is the main organ affected in patients with MDA5+ DM. IFN-κ, a member of the type I IFNs, is induced following viral infection or treatment of cells with double-stranded RNA or other IFNs, and IFN-κ signaling induces a set of genes via the type I IFN receptor. Unlike other members of the type I IFNs, IFN-κ is highly expressed in keratinocytes (LaFleur et al., 2001; Scarponi et al., 2006) and has been shown to play a pivotal role in the pathophysiology of skin involvement in systemic lupus erythematosus (Sarkar et al., 2018; Stannard et al., 2017). Functional studies on the role of MDA5 antibodies that may modulate inflammatory signaling pathways in patients affected by MDA5+ syndrome in the skin or in the lung may be interesting. Our study corroborates a recent publication (Zhang et al., 2019) and brings insights on the role of IFN-κ in the skin of patients with MDA5+. Our data support the potential role of keratinocytes and IFN-κ over IFN-α in the skin pathophysiology of MDA5+ DM.

Data availability statement

Datasets related to this article can be found at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128314

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: CC, RA, J-DB, HLB; Data Curation: CC, RA, MD, JP, AH-K, AJA; Formal Analysis: CC, RA, MD, MBat, JP, AH-K; Funding Acquisition: MBag, AB, J-DB, HLB; Investigation: CC, RA, MBat, JL-C, JW, SD, AJA, MM; Project Administration: CC, RA, J-DB, HLB; Resources: MBat, CL, MJ, AdM, LF, FC, DB, MBag, J-DB, JL-C, JW; Software: MD, AH-K; Supervision: MBag, AB, J-DB, HLB; Validation: CC, RA; Visualization: CC, RA, MBat; Writing - Original Draft Preparation: CC, RA; Writing - Review and Editing: CC, RA, MD, MBat, JP, AH-K, CL, MJ, AdM, LF, FC, DB, JL-C, JW, SD, AJA, MM, MBag, AB, J-DB, HLB.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.10.020.

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SUPPLEMENTARY MATERIALS AND METHODS

Patients

This study enrolled 27 patients with dermatomyositis (Supplementary Table S1) recruited from the dermatology department of Saint-Louis Hospital, Assistance-Publique Hôpitaux de Paris, Paris, France. Diagnosis was made according to the European Neuromuscular Center criteria (Hoogendijk et al., 2004; Sontheimer, 2002). No patient received immunosuppressive drugs before sample collection. Frozen sections of lesional skin were obtained from liquid frozen stored diagnostic samples. Punch biopsies of normal (n = 5) skin were obtained from healthy subjects through fresh plastic surgery waste and stored in RNA later until RNA extraction.

Microarray: skin samples and RNA extraction

From a lesional dermal-epidermal skin biopsy, 20 frozen sections, each 10-μm-thick, were taken (samples included optimal cutting temperature compound preserved biopsies at −80 °C). Total RNA was extracted from frozen tissue samples using the RNeasy Mini Kit Plus protocol (Qiagen, Hilden, Germany).

Healthy skin samples were prepared previously according to the following method: (i) single-use scalpel dissection into 2 × 2 × 2 mm fragments; (ii) immersion of the sample in lysis Buffer RLT (Qiagen) and beta-mercaptoethanol (10 μl/ml); and (iii) mechanical dissociation by oscillations (tissue lyser, Qiagen), parameterized at 50 Hz, on three sequences of 5 minutes.

RNA quality was assessed by microfluidic capillary electrophoretic migration via the Caliper Labchip GX II analyzer (PerkinElmer, Waltham, MA). RNA quality score was 4.3 ± 1.1 for dermatomyositis and 8.84 ± 0.2 for healthy donors.

Microarray analysis

Human genome U 2.0 gene chips (Affymetrix, Santa Clara, CA) were used for this study. After standardization according to the robust multiarray average method, quality control was performed by comparison between individuals of the average raw signal and the median absolute deviations. We excluded from this analysis non-coding RNAs. The bioinformatic used LIMMA for R (The R Foundation, Vienna, Austria). A first approach consisted of unsupervised hierarchical clustering. A second step performed a differential expression study between dermatomyositis and controls, according to the moderate Student test. The gene list and transcripts revealed were confronted with enrichment software in ontological and signaling functions, Molecular Signatures Database hallmark gene set collection/gene set enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathways. The pathways for which the false discovery rate *q*-value was < 0.05 were considered to be differentially enriched in a statistically significant manner. Gene nomenclature was apprehended via GeneBase. The results interpretation used the Transcriptome Analysis Console software suite (Affymetrix). The pathogenic pathways enrichment analysis used the Molecular Signatures Database platform. The analysis was ordered according to statistical significance (false discovery rate *q*-value < 0.05). Qlucore Omics Explorer was used to identify most differentially expressed genes and for the Venn diagram.

Quantitative real-time reverse transcriptase–PCR: skin samples, RNA extraction, cDNA synthesis, and PCR analysis

From a lesional dermal-epidermal skin biopsy, 10 formalin-fixed paraffin-embedded sections, each 10-μm-thick, were taken. Total RNA was automatically extracted from tissue samples using the Maxwell RSC RNA FFPE kit protocol (Promega, Charbonnières-les-bains, France). cDNA was synthesized from total RNA using a reverse transcription kit PrimerScript RT reagent Kit with gDNA eraser (Takara Bio Europe SAS, Saint-Germain-en-Laye, France). RNA quantity and quality assessment were performed with a Nanodrop instrument (Thermo Fisher Scientific, Waltham, MA).

The expression levels of six genes of the type I IFN signature and of the *IFNK* gene were tested by real-time quantitative PCR. Real-time quantitative PCR was carried out using the LightCycler 480 SYBR Green I Master (Roche, Basel, Switzerland). Primers used are the following: *ISG15* (ISG15_F: CGTGCTGGTGGTGGACAA; ISG15_R: CTCGTAGGTGCTGCTGCG), *IFIH1* (IFIH1_F: CCTCCTTCAGCCCACTCT; IFIH1_R: CAGCAGCAATCCGGTTTCT), *RSAD2* (RSAD2_F: CCTGGATGTTGGT GTAGAA; RSAD2_R:GCAGACAATG GCAGTTAC), and *CXCL10* (CXCL10_F: AGGTGCTATGTTCTTAGTGGATGT; CXCL10_R: GGAGGATGGCAGTG-GAAGT). *MX1*, *IFIT1*, and *IFNK* were designed and obtained from PrimerDesign (PrimerDesign, Chandler's Ford, UK). A LightCycler 480 II thermocycler (Roche) was used. PCR conditions were 95 °C for 5 minutes, followed by 45 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. At the end of the amplification reaction, a melting curve analysis was performed to confirm the specificity as well as the integrity of the PCR product by the presence of a single peak. Absence of cross-contamination and primer dimers was verified on a blank water control. The geometric mean of two reference genes (*TOP1* and *SDHA*) was used for normalization. The relative expression levels of mRNA were determined using the ΔΔCt formula; fold changes were calculated as $2^{-\Delta\Delta Ct}$. Only means of duplicates with a CV of <15% were analyzed.

Calculation of the type I IFN score

The mean and standard deviation (SD) of each of the six IFN-inducible genes (*ISG15*, *IFIH1*, *RSAD2*, *CXCL10*, *MX1*, and *IFIT1*) that are part of the signaling function of gene set enrichment analysis and Kyoto Encyclopedia of Genes and Genomes for the group of healthy donors (HDs) were used to calculate that gene's expression score for each study subject, defined as the number of HD SDs above the HD mean. Type I IFN score, represented by the sum of the scores for each of the four genes, was calculated for each subject. We considered a type I IFN score to be high if it fulfilled one of the two following criteria: (i) expression of at least two of the three IFN genes at a level ≤2 HD SDs above the HD mean, or (ii) expression of a single *IFN* gene at a level ≤4 HD

SDs above the HD mean (Kirou et al., 2004).

Immunohistochemistry

MxA expression was assessed on formalin-fixed paraffin-embedded skin tissue samples. An indirect immunoperoxidase method (Discovery/Roche Diagnostics) on 5-μm-thick tissue sections was performed using anti−human-MxA/Mx1 (R&D Systems, Biotechne brand, Lille, France) as the primary antibody. The secondary antibody was a rabbit monoclonal anti-mouse IgG1 (M1gG51-4, Abcam, Cambridge, United Kingdom) coupled with the anti-rabbit OmniMap detection kit (Roche). Systematic controls were the absence of primary antibody and use of an irrelevant primary antibody of the same isotype.

Human subject declaration

All studies have been approved by the appropriate institutional review boards and were conducted in accordance with current ethical and legal frameworks of the Declaration of Helsinki. Informed written consent was received from participants before inclusion in this study, according to our local ethic rules (CPP Paris 12).

Statistical analysis

Patient and control data were compared between groups using the nonparametric Mann-Whitney U-test for quantitative real-time PCR results. All analyses were carried out in GraphPad Prism (La Jolla, CA; version 8.0.1). Differences were considered significant at $P < 0.05$.

Data availability statement

Datasets related to this article can be found at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128314

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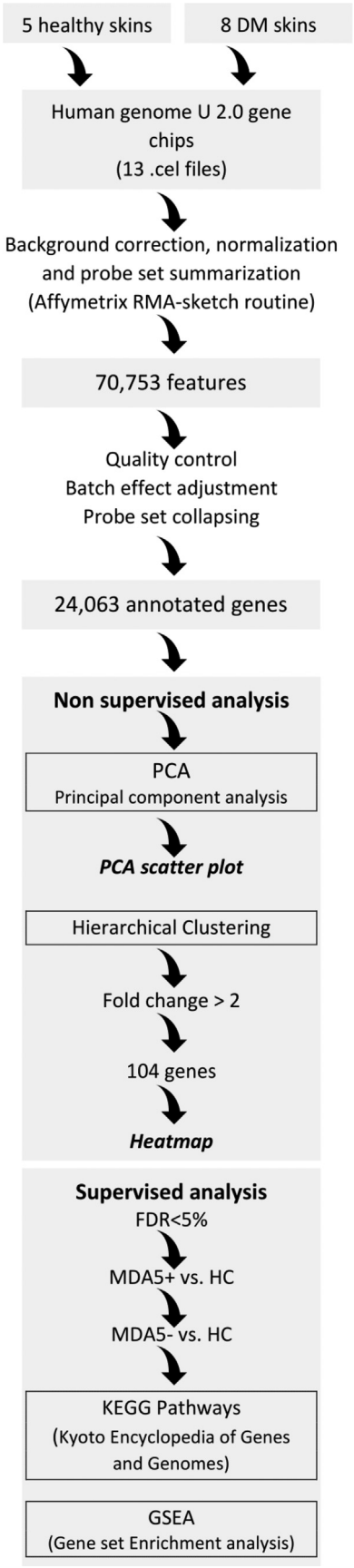
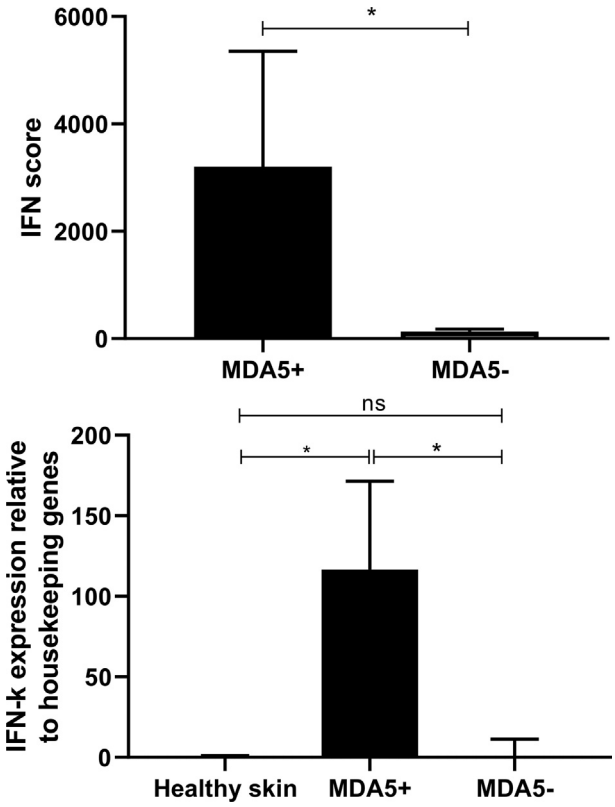


Supplementary Figure S1. Flowchart indicating the procedures followed in the transcriptomic analysis.
FDR, false discovery rate; GSEA, gene set enrichment analysis; HC, healthy control; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCA, principal component analysis.



Supplementary Figure S2. Type I IFN score and *IFNK* transcript in MDA5^{+} and MDA5^{-} DM skin. Total skin RNA from MDA5^{+} (n = 10) or MDA5^{-} (n = 9) DM paraffin-embedded skin samples was extracted and reverse transcribed for further mRNA expression analysis by real-time PCR. Histogram representation (mean and standard error of mean) of IFN score and IFN-κ expression are shown. *$P < 0.05$. DM, dermatomyositis; ns, not significant.

Supplementary Table S1. Clinical Characteristics of Enrolled Patients

									Skin manifestations								
Microarray	Age	Sex	Serology	Phenotype	ILD	Muscle	Neoplasia	Treatment	Gottron Papules	Hands dorsum erythema	Periungual erythema	Palm erythema	Eyelid lilac erythema	Face erythema	Ulceration	Palm erythema	Biopsy site
1	46	M	MDA5	CADM	RPILD	No	No	None	x	x	x			x	x	x	Papule of the forehead
2	50	F	MDA5	CADM	ILD	No	No	None	x	x		x			x		Erythematous papule of the back
3	48	F	MDA5	CADM	ILD	No	No	None	x	x		x			x	x	Erythematous papule of the neck
4	85	F	Negative	CADM	No	No	No	None						x			Erythematous papules of the face
5	55	F	Negative	CADM	No	No	No	None		x	x			x			Erythematous and scaly lesions of the arms
6	46	F	TIF1γ	CADM	No	No	No	None									Erythematous papules of the fingers
7	56	M	Negative	CADM	No	No	No	None		x	x			x			Papule of the thigh
8	71	F	Negative	CADM	ILD	No	No	None		x			x				Unknown
1	31	F	MDA5	CADM	No	No	No	None	x	x	x	x	x			xx	Erythematous papules of the finger
2	48	F	MDA5	CADM	ILD	No	No	None	x	x		x					Erythematous and scaly papules of the thigh
3	52	F	MDA5	CADM	ILD	No	No	None	x	x	x		x			x	Erythematous papules of the arm
4	22	F	MDA5	CADM	ILD	No	No	None				x	x			x	Erythematous papules of the neck
5	47	M	MDA5	CADM	RPILD	No	No	None	x	x				x	x		Papules of the forehead
6	23	M	MDA5	CADM	ILD	No	No	None		x			x		x	x	Unknown
7	58	M	MDA5	CADM	No	No	No	None	x	x	x		x			x	Erythema of the back
8	69	M	MDA5	CADM	No	No	Yes	None	x	x	x		x		x		Erythema of the neck

(*continued*)

Supplementary Table S1. Continued

									Skin manifestations								
Microarray	Age	Sex	Serology	Phenotype	ILD	Muscle	Neoplasia	Treatment	Gottron Papules	Hands dorsum erythema	Periungual erythema	Palm erythema	Eyelid lilac erythema	Face erythema	Ulceration	Palm erythema	Biopsy site
9	68	F	MDA5	CADM	RPILD	No	No	None	x	x	x	x			x	x	Erythematous papules of the finger
10	30	M	MDA5	CADM	No	No	No	None	x	x						x	Erythematous papules of the arm
11	43	M	Negative	CADM	No	No	No	None		x			x				Erythematous papules of the finger
12	55	F	Negative	DM	No	Yes	No	None		x			x				Panniculitis of the arm
13	77	F	Negative	DM	No	Yes	No	None						x			Erythema of the neck
14	85	F	Negative	CADM	No	No	No	None					x	x			Erythematous papules of the back
15	67	M	Mi2	DM	No	Yes	No	None	x				x	x			Erythema of the neck
16	58	F	Negative	CADM	No	No	No	None	x				x				Erythematous papules of the face
17	39	F	Negative	DM	No	Yes	No	None		x							
18	41	F	Negative	CADM	No	No	No	None	x		x		x				Erythematous papules of the finger
19	62	M	Negative	DM	No	Yes	No	None					x	x			Erythematous papules

Abbreviations: CADM, clinical amyopathic dermatomyositis; DM, dermatomyositis; F, female; ILD, interstitial lung disease; M, male; RPILD, rapidly progressive interstitial lung disease.

Supplementary Table S2. Histological Characteristics of Enrolled Patients for Microarray Analysis

Patient number	Serology	CD123[1] (% of mononucleated inflammatory cells)	Epidermic MxA[2] infiltration	Dermic MxA infiltration
1	MDA5	5	++	++
2	MDA5	0.5	++	++
3	MDA5	3	++	++
4	Negative	0.5	0	0
5	Negative	3	+	+
6	TIF1γ	3	0	0
7	Negative	3	0	0
8	Negative	N.A.	N.A.	N.A.

Abbreviation: N.A., not applicable; TIF1γ, transcriptional intermediary factor 1 gamma.
[1]CD123 (or IL3Ra) was considered as a marker of plasmacytoid dendritic cells.
[2]IFN-induced GTP-binding protein Mx1 was considered as a marker of type I IFN signature.