Materials and Methods

Patients

Skin specimens were obtained from the involved skin of 25 DM, 20 SLE, 3 psoriasis (PSO), 3 atopic dermatitis (AD), 10 lichen planus (LP) and 10 erythema exsudativum multiforme (EEM) patients. Control skin samples were obtained from the routinely discarded skin of 15 normal subjects (NS) undergoing skin graft.

The DM patients were diagnosed based on the criteria proposed by Bohan and Peter.[11-13] Patients with SLE fulfilled the criteria proposed by the American College of Rheumatology (ACR).[14] We randomly and retrospectively collected samples from patients who visited our department from April 2008 to March 2014. Among various cutaneous lesions of DM or SLE, erythematous eruptions mainly caused by interface dermatitis were included in this study, and samples with panniculitis only without erythema were excluded. This study was approved by the Ethics Review Committee in Kumamoto University (No. 1452). Written informed consent was obtained according to the Declaration of Helsinki.

Liquid chromatography-tandem mass spectrometry-based quantitative proteomic analysis

Analysis of liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed in accordance with the protocol provided by iTRAQ® analysis service (Filgen, Nagoya, Japan). Proteins were extracted from each skin sample utilising T-PER tissue protein extraction reagent (Pierce, Rockford, IL, U.S.A.) with protease inhibitor cocktail (Sigma-Aldrich St. Louis, MO, U.S.A.). The extracted proteins were condensed, reductive alkylated, and digested with trypsin. The peptides were desalted by Sep-Pak® light C18 cartridge (Waters, Milford, MA, U.S.A.) and were labelled with iTRAQ reagent-mutiplex assay kit (AB Sciex). The labelled peptides from each sample were mixed and separated into 8 fractions using caution exchange buffer pack (AB Sciex). The mass spectrometric analysis was performed in the AB Sciex triple TOF 5600 system and DiNa system. The values were normalized via global normalization based on the measured median ratios.[15]

RNA isolation and quantitative real-time polymerase chain reaction

RNA extraction and quantitative real-time polymerase chain reaction (PCR) were performed as described previously.[16]

Immunoblotting

Immunoblotting was performed by using antibodies for proteasome subunit beta type 9 (PSMB9)/LMP2 (clone1c4, LSBio, Seattle, WA, U.S.A.), VCAN GAT (Origene, Rockville, MD, U.S.A.), collagen I Goat-UNLB (Southern Biotech, Birmingham, AL, U.S.A.) or β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.).[17]

Measurement of collagen content

Collagen contents in paraffin-embedded skin sections were measured by quantitative micro-assay kit (Chondrex, Redmond, WA, U.S.A.) following the manufacturer's instructions.[18]

Immunohistochemical analysis

Immunohistochemistry was performed with primary antibodies for PSMB9, versican V1 or TGF-β2 (LSBio, Seattle, WA, U.S.A.).[19]

Cell cultures

Normal human epidermal keratinocytes, normal human dermal fibroblasts, and human immortalised keratinocytes cell line, HaCaT, were cultured as described previously.[19-21]

Transient transfection

siRNAs against PSMB9/LMP2 and control siRNA were purchased from Santa Cruz Biotechnology and Thermo, respectively. Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, U.S.A.) was used as the transfection reagent.[22]

Quantification of GAGs

The quantification of GAGs was performed in accordance with the protocol provided by Glycoscience Laboratory (Tokyo, Japan). For the quantification of hyaluronic acid (HA), after degreasing, dehydration, and drying, each skin sample was digested by collagenase (Seikagaku Co, Tokyo, Japan) and actinase E (Kaken Seiyaku, Tokyo, Japan). Finally, the digests were boiled to extract HA. Then, HA polymer (Calbiochem, Cambridge, MA, U.S.A.) was added into each well of a 96-well amine-conjugated microtitre plate (Sumitomo Bakelite, Tokyo, Japan), followed by incubation with sodium cyanoborohydrate solution and diluted block ace solution (DS Pharma Biomedical, Osaka, Japan). Diluted samples were added into the wells followed by addition of biotinylated HA-binding protein solution (Hokudo, Sapporo,

Japan). After washing, samples were incubated with horseradish peroxidase (HRP)-conjugated streptavidin solution (Calbiochem) for 1 hour. TMB peroxidase substrate was added into each well, and incubated for 20 minutes. Absorbance at 450 nm was measured by using MTP-300 Microplate Reader (Corona Electric, Tokyo, Japan).

Next, for the quantification of chondroitin sulfate (CS), the samples were treated with hyaluronidase (Amano enzyme, Nagoya, Japan). High-performance liquid chromatography (HPLC) analysis was used to confirm that HA was completely removed by analysis. CS in each sample was then digested into disaccharides with chondroichinase AC2 (Seikagagu Co.). The digests were collected via centrifuge and analysed by HPLC analysis.

Lastly, CS in samples was completely removed by chondroichinase ABC (Seikagagu Co.), which was confirmed by HPLC. Heparan sulfate (HS) in each sample was digested into disaccharides with hepatirinase (Seikagagu Co.) for the quantification of HS by HPLC.

Statistical analysis

Data presented as bar graphs are the means+standard deviation of at least three experiments. Statistical analysis was conducted by using the Mann-Whitney *U* test for the comparison of medians.

Results

Protein expression profiles in DM skin

First, quantitative proteomic analysis was performed utilising LC-MS/MS in order to determine whether molecules upregulated or downregulated in the skin with DM. Protein samples were obtained from 2 DM skin samples (DM1 and DM2) and 2 control skin samples of normal subjects (NS1 and NS2). The clinical features of the DM patients are shown in Supplemental Table. The NS samples were matched with the DM samples for age, gender, and biopsy site. The expression profiles of 3,268 proteins were compared between the DM and NS skin, and expression ratios >1.5 or $\leqq$0.67 were regarded as upregulated or downregulated, respectively. We identified 61 molecules whose expressions in both DM samples were upregulated or downregulated in comparison with both of the NS samples (Table 1). Twenty-seven of the 61 proteins were upregulated in DM skin, while 34 proteins were downregulated.

We selected 19 genes, which may be associated with the pathogenesis of DM (e.g., involved in immunity, antigen presentation, or IFN pathway), and their mRNA levels were

analysed by real-time PCR with an additional number of samples (randomly chosen 8 NS and 8 DM skin, Fig. 1a) to validate the LC-MS/MS analysis. Among the 19 genes, consistent with the LC-MS/MS results, versican variant 1 (versican V1), SPRR1B, THBS1, PSMB9, EIF1AX, and CTSL1 were significantly upregulated in DM skin compared to NS skin, whereas PIP, SPTAN1, COL1A2, COL1A1, and PI16 were significantly downregulated in DM skin. The biopsy sites (e.g., face, arm, hand), age, or gender did not affect the expression levels of these molecules.

Antibodies for aminoacyl-tRNA synthetase (ARS) have recently attracted attention as disease-specific/associated antibodies of myositis. However, the expressions of ARS-related genes including histidyl-tRNA synthetase (HARS) or tryptophanyl-tRNA synthetase (WARS) showed no significant differences between DM and NS based on real-time PCR (Fig. 1b) and LC-MS/MS results.

Expression of PSMB9, versican V1 and type I collagen in DM and SLE skin

Among these upregulated or downregulated molecules in DM skin, we first focused on PSMB9, an immunoproteasome subunit, because Nakajo-Nishimura syndrome, which is caused by PSMB8 mutations, demonstrates symptoms similar to those of DM and SLE (e.g., facial erythema, pernio-like eruptions, and myositis).[23] Real-time PCR using additional numbers of skin samples of DM (n=25), SLE (n=20) and NS (n=15) showed that PSMB9 mRNA levels were significantly upregulated in both DM and SLE skin compared to NS skin (Fig. 2a). Although we analysed the correlation between PSMB9 levels and biopsy sites (e.g., face, arm, hand), the types of cutaneous manifestations (e.g., heliotrope, Gottron's papule, malar rash, discoid lupus erythematosus), age, and gender, these factors did not significantly affect the PSMB9 levels. PSMB9 expression was not altered in PSO and AD, which were sometimes differential diagnoses of DM/SLE. Furthermore, to confirm the possibility that the PSMB9 upregulation was elicited by the pathophysiological mechanism of DM/SLE, samples of the other interface dermatitis were included, but PSMB9 mRNA levels in LP or EEM skin were not increased compared to NS skin. Thus, PSMB9 overexpression was rather specific to DM and SLE skin. PSMB8 mRNA levels tended to be elevated in DM and SLE skin, but no statistically significant differences were observed (Fig. 2b).

Next, we determined the levels of versican V1, a well-known core protein which binds with GAGs and forms proteoglycans. Dermal proteoglycan deposition is a common histopathological characteristic of DM and SLE skin. The mRNA levels of versican V1 were significantly upregulated in both DM and SLE skin compared to NS, AD and LP (Fig. 2c). In

contrast, we did not find any significant differences in the expression of versican V3, a variant that lacks GAGs attachment sites, or aggrecan, another known core protein, between DM and NS by real-time PCR (Fig. 1b).

Type I collagen is the most abundant extracellular matrix in the dermis of human skin and consists of COL1A1 and COL1A2. The mRNA levels of COL1A1 and COL1A2 were significantly downregulated in both DM and SLE skin compared to those in NS, AD and LP (Fig. 2d, e). Consistently, protein levels of type I collagen in vivo were decreased in both DM and SLE skin compared to NS skin and scleroderma skin, a positive control characterised by collagen overexpression (Fig. 2f). PSMB9 protein was increased in DM and SLE skin, but not scleroderma skin. We also performed quantitative analysis of collagen content in paraffin-embedded sections of randomly chosen 8 DM and 6 NS skin. A significant decrease in collagen level was observed in DM skin (Fig. 2g).

The localisation of increased PSMB9 and versican V1 in DM and SLE skin

To clarify the localisation of increased PSMB9 and versican V1 levels in DM and SLE skin, immunochemical staining of these molecules was performed utilizing skin specimens from DM (n=16), SLE (n=8) and NS (n=8). We found that PSMB9 expression was increased in the epidermis of both DM and SLE skin compared to NS skin (Fig. 3a). No apparent differences in PSMB9 staining of infiltrated cells were found between the dermis of DM, SLE or NS. We also tested other inflammatory diseases, including PSO, AD, LP, and EEM as well as vasculitis, lymphoma, and sarcoidosis, but PSMB9 expression was not increased in the epidermis of these diseases (data not shown). On the other hand, versican V1 expression was found between the collagen bundles of the dermis in DM and SLE skin, but not in NS skin (Fig. 3b).

Effects of IFN on the expression of PSMB9, collagen and versican V1

Our results suggested that PSMB9 was increased in the epidermis of DM and SLE, while type I collagen was decreased and versican V1 was increased in their dermis. Next, we determined whether these changes in DM and SLE skin were caused by IFN signaling. Immunoblotting showed that IFN-α and IFN-γ induced PSMB9 expression in cultured epidermal keratinocytes (Fig. 3c). Furthermore, IFN-α and IFN-γ reduced type I collagen expression in cultured dermal fibroblasts, while they did not affect versican V1 expression.

Therefore, both IFN-α and IFN-γ directly controlled the expressions of PSMB9 and type I collagen, but not versican V1.

These data led us to determine the detailed mechanism of versican overexpression in the dermis of DM and SLE skin. We hypothesised that PSMB9 is induced by IFN in the epidermis, which stimulates versican V1 expression in the dermis. Previous reports indicated that several cytokines (e.g., TGF-β2 and TGF-β3) stimulate versican expression.[24] When PSMB9 was knocked down by PSMB9 siRNA in keratinocytes (Fig. 4a), versican expression was not affected. On the other hand, PSMB9 knockdown led to the significant suppression of TGF-β2 and TGF-β3, but not TGF-β1 (Fig. 4b). Taken together, our hypothetical model is that increased PSMB9 due to IFN signaling induces TGF-β2/3 in epidermal keratinocytes of DM/SLE, which results in the stimulation of versican synthesis in dermal fibroblasts (Fig. 5). Consistently, TGF-β3 mRNA levels in vivo were significantly upregulated in both DM and SLE skin compared to NS skin and skin of other diseases (Fig. 4c). Of note, TGF-β2 mRNA levels were upregulated only in DM skin. Immunohistochemical findings confirmed that TGF-β2 protein expression was increased only in the epidermis of DM, but not SLE skin (Fig. 4d).

GAGs analysis in DM and SLE skin

We also determined the amount of GAGs in DM and SLE skin. The CS levels were significantly increased in SLE skin compared to NS skin (Fig. 6a). On the other hand, no significant differences were found in the amount of HA or HS between the skin of DM, SLE and NS.

Next, the composition of 6 disaccharides of HS was quantitated by HPLC. We found that the ΔDiHS-diS1 levels were significantly increased only in DM skin compared to SLE and NS (Fig. 6b).

We then determined the mechanism underlying the increased levels of ΔDiHS-diS1 only in DM skin. The stimulation of normal human dermal fibroblasts with TGF-β2 resulted in the significant increase of ΔDiHS-diS1 secretion levels (Fig. 6c). Accordingly, the difference of ΔDiHS-diS1 levels between DM and SLE skin may be associated with the difference of TGF-β2 expression between them.

Discussion

This is the first report of a proteomic analysis using skin samples of DM patients. In the present study, we identified common molecular changes in DM skin and SLE skin and specific molecular changes to DM skin.

Based on the LC-MS/MS results, we focused on the increase of PSMB9, a subunit of immunoproteasome, as common molecular change in DM and SLE skin because of the similarity between Nakajo-Nishimura syndrome (caused by mutations of PSMB8, another immunopreteasome subunit) and DM/SLE.[23] Proteasomes are components of the ubiquitin-proteasome system (UPS), where immunoproteasomes degrade intracellular proteins into peptide fragments, improves bindings of the fragments to major histocompatibility complex (MHC) class I molecules, and induces immune responses effectively. The UPS activation by PSMB overexpression may play a role in the pathogenesis of autoimmune diseases such as DM and SLE. Consistently, bortezomib, an inhibitor of PSMB8, is found to be effective for SLE patients and currently under clinical trial.[25]

In addition, PSMB9 is encoded by MHC class II region, and recent genome-wide association studies (GWAS) indicated MHC as the major genetic region associated with DM or SLE.[26,27] Furthermore, PSMB9 is also known as the target gene of IFN.[28,29] Type I IFN signature in DM and SLE has been well investigated. Meanwhile, several studies have also suggested the involvement of IFN-γ in these diseases.[30,31] Taken together, PSMB9 is a key molecule associated with both MHC and IFN in DM and SLE. PSMB9 overexpression is highly specific to these two diseases, and therefore it may be useful as a diagnostic tool and new therapeutic target.

Collagens and proteoglycans are the main components of the extracellular matrix in skin dermis. Versican is a core protein that mainly interacts with HA and CS, and controls mucin deposition. Furthermore, previous studies suggested that versican also binds to HS indirectly.[32-34] Versican expression was increased, while type I collagen expression was decreased in DM and SLE skin compared to NS skin. As the mechanism of increased cutaneous mucin deposition in DM and SLE, versican upregulation and collagen downregulation may accelerate it.

Chang *et al.* reported that both HA and CS were significantly elevated in discoid LE (DLE) by specific stains, while DM lesional dermis accumulated mainly CS but not HA.[35] Kim and Werth also revealed increased accumulation of chondroitin-4-sulfate in DLE and DM lesions compared to control skin by immunohistochemical analyses.[36] Our study is

the first to focus on HS in these diseases, and identified TGF-β2-ΔDiHS-diS1 pathway as the specific molecular changes in DM skin. TGF-β2 expression was increased only in the epidermis of DM skin, and TGF-β2 induced ΔDiHS-diS1 in cultured dermal fibroblasts. The difference in this pathway may also be useful to distinguish DM from SLE. Furthermore, because different GAGs species have distinct immunomodulatory effects, the TGF-β2-ΔDiHS-diS1 pathway may play a role in the difference between the skin features in DM and SLE (e.g. predilection sites). Further studies are needed to identify upstream events of TGF-β2 increase in DM skin in the future.

Of note, PSMB9 knockdown resulted in the significant suppression of TGF-β2 and TGF-β3 expressions, but not TGF-β1 expression. In spite of their structural similarities, previous studies have shown that TGF-β1 and TGF-β2/3 can be differentially regulated: for example, Mężyk-Kopeć *et al.* reported that silencing of a disintegrin and metalloprotease 17 (ADAM17) induced the levels of TGF-β2 and TGF-β3 in human dermal lymphatic endothelial cell line, but did not affect the TGF-β1 expression.[37] Furthermore, Olave *et al.* revealed that endothelin-A receptor antagonism up-regulated TGF-β2 and TGF-β3 synthesis, but not TGF-β1 in mice.[38] Clarification of detailed mechanism of TGF-β2/3-specific regulation by PSMB9 is necessary in the future. As the limitation of this study, the study design may be rather story-oriented, and further studies are needed to prove our hypothetical model.

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Figure legends

Figure 1. Comparison of gene expression between dermatomyositis skin and normal skin

(a,b) Total RNA was extracted from involved skin tissues of dermatomyositis (DM) patients and control skin of normal subjects (NS). A subset of samples (8 DM skin and 8 NS skin) was randomly chosen from 25 DM skin and 15 NS skin. mRNA expression of indicated molecules was analyzed by real-time PCR. Bars show the means. The mean values in NS were set at 1. *P <0.05, **P <0.01, ***P <0.001.

Figure 2. PSMB9 expression in DM skin

(a) Total RNA was extracted from skin tissues derived from NS (n=15) and patients with DM (n=25), systemic lupus erythematosus (SLE, n=20), psoriasis (PSO, n=3), atopic dermatitis (AD, n=3), lichen planus (LP, n=10) and erythema exsudativum multiforme (EEM, n=10). PSMB9 mRNA expression was analyzed by real-time PCR. Bars show the means. The mean value in NS was set at 1. *P<0.05.

(b) Total RNA was extracted from skin tissues derived from DM (n=9), SLE (n=5) and NS (n=10): A subset of samples was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. PSMB8 mRNA expression was analyzed by real-time PCR.

(c-e) Total RNA was extracted from skin tissues derived from NS (n=15) and patients with DM (n=22) and SLE (n=20): A subset of samples was randomly chosen from 15 NS skin, 25 DM skin and 20 SLE skin. mRNA expression of indicated genes was analyzed by real-time PCR. Samples of 3 AD patients and 10 LP patients were also included as disease controls.

(f) Cell lysates obtained from skin tissues of indicated diseases were subjected to immunoblotimg with antibodies for PSMB9 or type I collagen. β-actin was used as the loading control. The 2 representative results for 5 samples of each disease randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin are shown. Two scleroderma (SSc) samples were also included as a disease control.

(g) Relative collagen content in paraffin-embedded sections of NS (n=6) and DM skin (n=8) was determined using the assay kit: A subset of samples was randomly chosen from 25 DM skin and 15 NS skin. *P<0.05.

Figure 3. The effects of interferon (IFN) on the expression of PSMB9, collagen and versican V1

(a) Paraffin sections were subjected to immunohistochemical analyses for PSMB9. *(left)* NS skin, *(middle)* DM skin, and (*right*) SLE skin. Original magnification x200 (upper panels) and x400 (lower panels). A subset of samples for each disease (n=15) was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. The representative results were shown.

(b) Paraffin sections were subjected to immunohistochemical analysis for versican V1. *(left)* NS skin, *(middle)* DM skin, and (*right*) SLE skin. Original magnification x200 (upper panels) and x400 (lower panels). A subset of samples for each disease (n=8) was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. The representative results were shown.

(c) Cultured normal human epithelial keratinocytes were incubated in the presence or absence of IFN-α *(left)* or IFN-γ *(right)* for 48 hours. Cell lysates were subjected to immunoblotting using antibody for PSMB9. β-actin was used as the loading control.

(d) Cultured normal human dermal fibroblasts were stimulated with indicated dose of IFN-α or IFN-γ for 72 hours. Cell lysates were subjected to immunoblotting using antibodies for type I collagen or versican V1. β-actin was used as the loading control.

Figure 4. Functional analysis of PSMB9 in keratinocytes

(a) HaCaT cells were transfected with control or PSMB9 siRNA. After 48 hours, cell lysates were obtained and subjected to immunoblotting with antibodies for PSMB9 or versican V1. β-actin was used as the loading control.

(b) HaCaT cells were transfected with control or PSMB9 siRNA, and total RNA was extracted after 24 hours. mRNA expression levels of indicated genes were determined by real-time PCR (n=3). The mean values in control cells were set at 1. *P <0.05.

(c) Total RNA was extracted from skin tissues derived from NS (n=5) and patients with DM (n=5) and SLE (n=5): A subset of samples was randomly chosen from 15 NS skin, 25 DM skin and 20 SLE skin. Samples of psoriasis (PSO, n=3), atopic dermatitis (AD, n=3), lichen planus (LP, n=10) and erythema exsudativum multiforme (EEM, n=10) were also included as the disease controls. mRNA levels of TGF-β2 and TGF-β3 were analyzed by real-time PCR. Bars show the means. *P<0.05.

(d) Paraffin sections were subjected to immunohistochemical analysis for TGF-β2. *(left)* NS skin, *(middle)* DM skin, and (*right*) SLE skin. Original magnification x200 (upper panels) and x400 (lower panels). A subset of samples for each disease (n=8) was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. The representative results were shown.

Figure 5. Our hypothetical model

IFN signaling in DM and SLE skin reduces the expression of collagen, but not versican V1 in dermal fibroblasts. IFN also stimulates PSMB9 expression and subsequently induces TGF-β2 or β3 in keratinocytes, which may increase versican V1 expression in dermal fibroblasts. According to previous reports, TGF-β2 and -β3 are not likely to affect on collagen expression in dermal fibroblasts.

Figure 6. Glycosaminoglycan analyses in DM, SLE and NS skin

(a) Hyaluronic acid (HA), chondroitin sulfate (CS) and heparin sulfate (HS) were extracted from skin tissues of DM (n=4), SLE (n=3) and NS (n=3): A subset of samples was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. The amount of each content /1mg of sample was shown on the ordinate. Bars show the means. *P<0.05.

(b) High performance liquid chromatography (HPLC) for various types of disaccharides composing HS was performed using skin tissues of DM (n=4), SLE (n=3) and NS (n=3). A subset of samples was randomly chosen from 25 DM skin, 20 SLE skin and 15 NS skin. The amount of each content corrected by total amount of HS was shown on the ordinate. *P<0.05.

(c) Normal human dermal fibroblasts were incubated in the presence or absence of TGF-β2 for 72 hours (n=3). HS was extracted from culture media, and was subject to HPLC for ΔDiHS-diS1. The results were normalized for cell numbers. *P<0.05.

Table 1. Comparison of protein expression between DM and normal skin determined by LC-MS/MS.

Gene symbol	Protein	DM1 /NS1	DM2 /NS2	DM1 /NS2	DM2 /NS1
DCD	Dermcidin	0.19	0.28	0.50	0.11
MAP1A	Microtubule-associated protein 1A	0.21	0.41	0.58	0.15
PIP	Prolactin-inducible protein	0.26	0.35	0.44	0.20
SPTAN1	Isoform 2 of Spectrin alpha chain, brain	0.30	0.52	0.59	0.27
GSTM2	Glutathione S-transferase Mu 2	0.36	0.30	0.29	0.38
COL1A2	Collagen alpha-2(I) chain	0.36	0.28	0.42	0.24
LONRF3	Isoform 3 of LON peptidase N-terminal domain and RING finger protein 3	0.42	0.21	0.49	0.18
SFRP1	Secreted frizzled-related protein 1	0.43	0.56	0.62	0.39
GIT1	GTPase-activating protein	0.43	0.44	0.41	0.46
COL1A1	Collagen alpha-1(I) chain	0.45	0.35	0.53	0.29
CKMT1	Isoform 1 of Creatine kinase U-type, mitochondrial	0.45	0.56	0.55	0.46
NOL10	Nucleolar protein 10	0.46	0.62	0.57	0.50
GSTT1	Glutathione S-transferase theta-1	0.47	0.63	0.67	0.44
CAMLG	Calcium signal-modulating cyclophilin ligand	0.47	0.48	0.46	0.49
PI16	Isoform 1 of Peptidase inhibitor 16	0.47	0.49	0.49	0.48
EPHX2	Epoxide hydrolase 2, cytoplasmic	0.48	0.57	0.55	0.50
GPLD1	Isoform 1 of Phosphatidylinositol-glycan-specific phospholipase D	0.49	0.19	0.26	0.36
EFCAB4B	EF-hand calcium-binding domain-containing protein 4B	0.51	0.58	0.47	0.63
RNF214	RING finger protein 214	0.53	0.56	0.46	0.65
GBAS	Protein NipSnap homolog 2	0.54	0.61	0.55	0.60
CCDC80	Isoform 1 of Coiled-coil domain-containing protein 80	0.55	0.39	0.59	0.36
GSTM1	Isoform 1 of Glutathione S-transferase Mu 1	0.56	0.45	0.52	0.48
PEBP1	Phosphatidylethanolamine-binding protein 1	0.56	0.55	0.61	0.50
APOH	Beta-2-glycoprotein 1	0.58	0.45	0.50	0.52

WDR16	Isoform 3 of WD repeat-containing protein 16	0.59	0.35	0.36	0.58
HSPA14	Heat shock 70 kDa protein 14	0.61	0.42	0.41	0.63
NDRG3	N-myc downstream-regulated gene 3 protein	0.62	0.33	0.34	0.62
CD109	Isoform 3 of CD109 antigen	0.62	0.46	0.56	0.51
HEBP2	Heme binding protein 2 (Fragment)	0.63	0.30	0.35	0.54
-	Conserved hypothetical protein	0.64	0.62	0.62	0.63
FLAD1	Flavin adenine dinucleotide synthetase 1	0.65	0.44	0.48	0.59
FBLN1	Isoform D of Fibulin-1	0.66	0.51	0.60	0.55
COL3A1	Isoform 1 of Collagen alpha-1(III) chain	0.67	0.46	0.63	0.48
IARS2	Isoleucyl-tRNA synthetase, mitochondrial	0.67	0.42	0.47	0.59
LOC402160	Uncharacterized protein	1.51	2.67	2.43	1.66
UGT2A3	UDP-glucuronosyltransferase 2A3	1.52	2.34	1.70	2.10
EIF2AK2	Interferon-induced, double-stranded RNA-activated protein kinase (Fragment)	1.53	1.61	1.50	1.65
-	V1-17 protein	1.55	2.79	1.65	2.62
CD209	Isoform 10 of CD209 antigen	1.57	2.58	2.48	1.63
ERAP2	Endoplasmic reticulum aminopeptidase 2	1.57	1.59	1.26	1.99
MANF	Mesencephalic astrocyte-derived neurotrophic factor	1.61	1.74	1.52	1.84
RPS10-NUDT3	RPS10-NUDT3 protein	1.63	1.71	1.67	1.68
FAM21C	Isoform 3 of WASH complex subunit FAM21C	1.69	2.06	2.18	1.59
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3	1.73	2.10	2.31	1.57
PF4	Platelet factor 4	1.74	1.85	1.76	1.83
CCPG1	Isoform 4 of Cell cycle progression protein 1	1.77	1.98	1.80	1.95
ARPC1B	Actin-related protein 2/3 complex subunit 1B	1.78	1.97	1.72	2.04

EIF3M	Eukaryotic translation initiation factor 3 subunit M	1.80	1.64	1.55	1.91
VCAN	Isoform V1 of Versican core protein	1.81	2.30	2.49	1.67
SPRR1B	Cornifin-B	1.88	1.60	1.92	1.56
THBS1	Thrombospondin-1	1.95	1.62	1.76	1.79
CCT7	Chaperonin containing TCP1, subunit 7	1.98	1.56	1.70	1.82
MAN1A1	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	2.09	1.53	1.90	1.68
STAT1	Isoform Alpha of Signal transducer and activator of transcription 1-alpha/beta	2.46	2.02	2.05	2.41
PSMB9	Isoform LMP2.S of Proteasome subunit beta type-9	2.57	2.77	2.51	2.83
EIF1AX	Eukaryotic translation initiation factor 1A, X-chromosomal	3.06	2.12	3.29	1.97
TYMP	Thymidine phosphorylase	3.35	1.50	1.53	3.28
HLA-B	HLA class I histocompatibility antigen, B-54 alpha chain	3.92	1.55	1.60	3.80
CTSL1	Cathepsin L1	4.25	1.92	2.36	3.46
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	4.25	1.71	2.42	3.00
GBP1	Interferon-induced guanylate-binding protein 1	5.44	1.59	1.65	5.25

Protein samples were extracted from DM skin (n=2) and normal skin samples (NS, n=2), and were analyzed by LC-MS/MS. Proteins whose expression ratio of DM1/NS1, DM2/NS2, DM1/NS2 and DM2/NS1 was always >1.5 or ≦0.67 are listed.

Figure 1

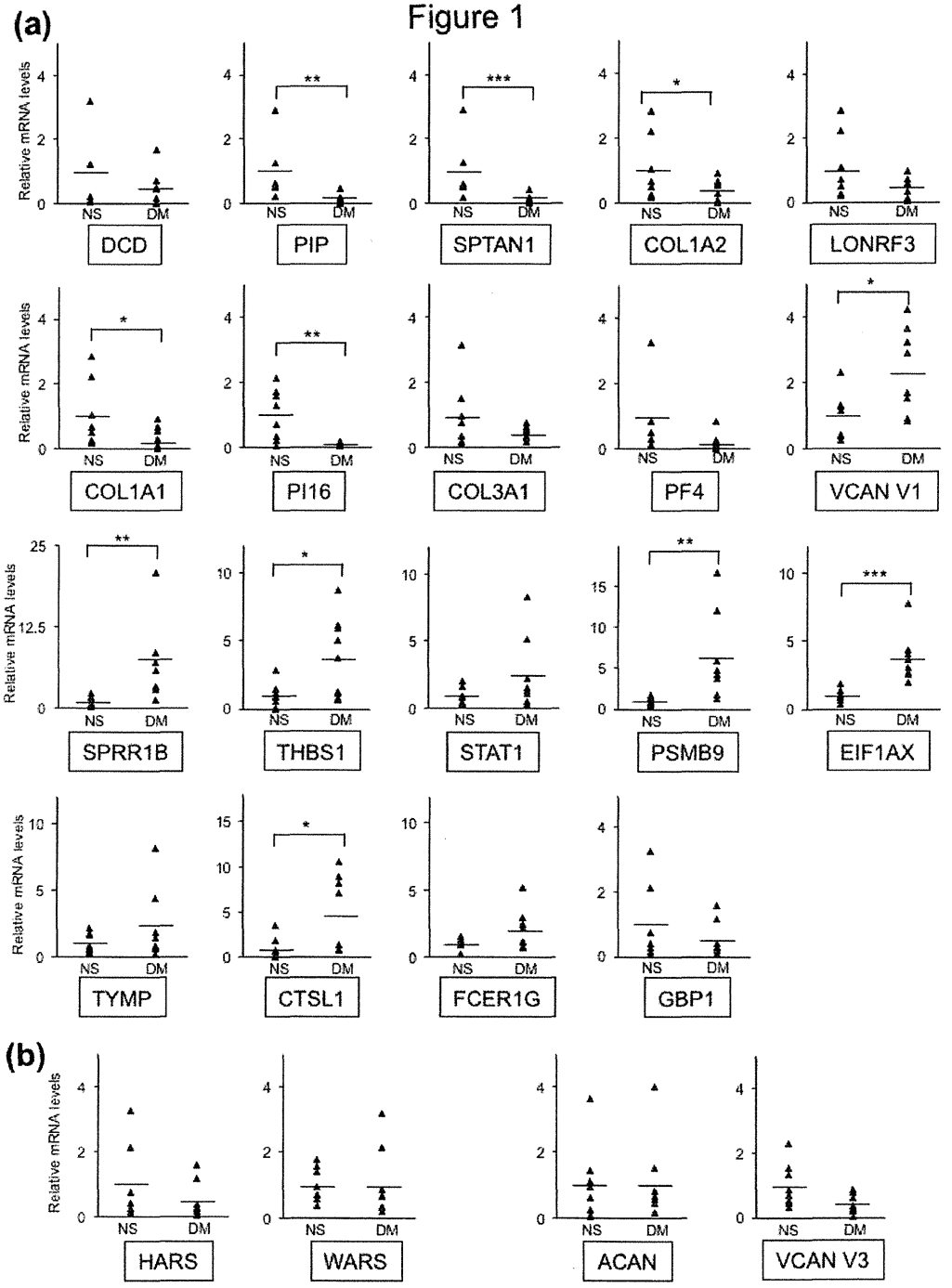

Figure 2