Gene expression profile of inflammatory myopathy with malignancy is similar to that of dermatomyositis rather than polymyositis

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Abstract

Objective An association has been reported between inflammatory myopathies (IIMs), which include polymyositis (PM) and dermatomyositis (DM), and malignancy, and the concept of cancer-associated myositis (CAM) was recently proposed. Herein, we attempted to determine the features and etiologies of these myopathies.

Methods We analyzed gene expression levels via microarray and real-time quantitative reverse transcription polymerase chain reaction to identify genes that were specifically upregulated or downregulated with suspected inflammatory involvement and verified the microarray data via immunohistochemical (IHC) analysis in additional cases.

Patients We selected 14 patients with the following conditions: PM without malignancy (n = 3), DM without malignancy (n = 3), CAM (n = 3), and Controls (no pathological changes or malignancy; n = 5).

Results PM was distinct from DM and CAM in a clustering analysis and exhibited the highest numbers of overexpressed genes and specific pathologies in a gene ontology analysis. IHC analysis confirmed the gene expression results.

Conclusions PM was associated with severe inflammatory pathological findings, primarily in the cell-mediated immune system. DM and CAM exhibit similarities in gene expression and IHC results, which suggest that humoral immunity is the main etiology for both myopathies, indicating the importance of cancer screening in patients with IIMs, particularly DM.

Key Words: Polymyositis, dermatomyositis, cancer-associated myositis, gene expression, immunohistochemistry

Introduction

Inflammatory myopathies (IIMs), which include polymyositis (PM) and dermatomyositis (DM), comprise a heterogeneous group of disorders characterized by muscle inflammation and weakness (1-4). The criteria developed by Bohan and Peter emphasize the clinical features of these disorders, particularly the presence or absence of a rash (1,2). However, many other studies have revealed the etiological differences between DM and PM. According to the criteria published by Dalakas and Holfield, specific pathological findings are required for a definitive diagnosis (5). Accordingly, various criteria, each with a particular specificity and sensitivity, have been proposed to classify PM and DM (6). Specifically, PM is assumed to involve an antigen-specific, cell-mediated immune response, whereas DM is thought to involve an autoimmune, humoral immune-mediated microangiopathy (7), although the antigens and triggers of these autoimmune responses remain unknown.

The association between IIMs, particularly DM, and malignancy has been well described (8-13). According to the first meta-analysis conducted to preliminarily confirm this association, malignancy was found to occur in 25% and 10%−15% of adult patients with DM and PM, respectively, within 0−5 years of disease onset (14).

In a recent meta-analysis study, the pooled risk ratios or standardized incidence ratios for patients with PM, DM, PM/DM relative to the general population were 1.62 (95% confidence interval: 1.19–2.04), 5.50 (4.31–6.70), and 4.07 (3.02–5.12), respectively (15). Additionally, many reports have described cancer-associated myopathy (CAM) (16-19). This disorder is thought to arise in response to the common expression of autoantigens on both cancer and muscle tissues, but the etiological mechanism remains unclear (20, 21). Because the pathology of CAM has not been established and many

cases of IIM do not fulfill the criteria described by Dalakas and Holfield, it is difficult to diagnose PM, DM, and CAM from pathological muscle findings alone.

Microarray techniques can be used to define clinically significant disease subtypes via molecular profiling of a tissue from a quantitative list of relative gene expression levels. This profile may serve as a ''signature'' of large-scale gene expression. In this study, we used microarray techniques and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) to analyze the characteristics of PM, DM, and CAM and confirmed our findings via immunohistochemical (IHC) analysis.

Patients and Methods

Patients

This retrospective study was approved by the ethical committee of Nagoya University. We retrospectively evaluated patients who had undergone muscle biopsy. The enrolled patients were divided into four groups according to their condition: PM, DM, CAM, and Controls. All patients with IIMs fulfilled the Bohan and Peter criteria for definite or probable IIM. Patients with PM and DM had no history of malignancy before the muscle biopsy and passed an interval of >3 years after biopsy without a diagnosis of malignancy. In patients with CAM, malignancy was diagnosed concurrently with the onset of myopathy. None of the patients had received any treatment, including immunotherapy, prior to muscle biopsy. The Control patients had undergone muscle biopsy that suspected some myopathies. However, they did not present with high plasma creatine kinase levels or any inflammatory changes evident in their muscle biopsies (Table 1).

Analytical approach.

Initially, we constructed gene expression profiles using microarray techniques and analyzed the gene ontology (GO) of each profile. According to our gene expression analysis, we verified the genes with expected inflammatory and pathological involvement using real-time qRT-PCR and IHC techniques. Table 1 presents the clinical data of 14 patients (PM, 3 cases; DM, 3 cases; CAM, 3 cases; and Control, 5 cases) whose specimens were subjected to microarray and real-time qRT-PCR analysis. In the microarray analysis, the enrolled PM and DM patients fulfilled both the Bohan ande Peter criteria and the Dalakas and Holfield criteria. The criteria developed by

Bohan and Peter for IIM were found to exhibit extremely poor specificity (29%),

whereas the criteria published by Dalakas and Holfield in 2003 exhibited high

specificity (99%) and sensitivity (77%) (22). Accordingly, we used the latter criteria to

identify patients for our microarray analysis and assumed that the selected patients in

both the PM and DM groups were relatively homogeneous.

   We enrolled additional patients for IHC gene expression verification of the results of

microarray and real-time qRT-PCR analyses. The patients enrolled for IHC analysis

were as follows: 14 patients with PM (4 men, 10 women; mean age, 63 years), 13

patients with DM (13 men, 9 women; mean age, 53 years), 25 patients with CAM (13

men, 12 women; mean age, 60 years), and 11 Control patients (7 men, 4 women; mean

age, 43 years). In the CAM group, the following malignancies were observed: lung

cancer (n = 6), gastric cancer (n = 5), breast cancer (n = 3), malignant lymphoma (n = 3),

tongue cancer (n = 1), laryngeal cancer (n = 1), esophageal cancer (n = 1),

hepatocellular carcinoma (n = 1), bile duct cancer (n = 1), uterine corpus cancer (n = 1),

ovarian cancer (n = 1), and prostate cancer (n = 1).

Gene expression analysis

RNA extraction

   Frozen muscle tissue samples (50–100 mg) were homogenized in 1 mL of ISOGEN

(Nippon Gene, Tokyo, Japan). Subsequently, 0.2 mL of chloroform was added to each

sample, followed by vigorous shaking for 15 s and centrifugation for 15 min at 4ºC.

Next, 0.5 ml isopropanol was added to each sample, followed by another centrifugation

step for 15 min at 4ºC. Isopropanol and 70% ethanol were used for RNA precipitation.

We measured RNA concentrations using a NanoDrop ND-1000 spectrophotometer

(Thermo Scientific, Waltham, MA, USA), and evaluated the quality of total RNA using

an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Microarray analysis

We used a SurePrint G3 Human GE microarray $8 \times 60k$ (Agilent Technologies) to

evaluate gene expression profiles. The SurePrint G3 microarray, which contains 27,958

Entrez gene targets and 7,419 long intergenic non-coding RNAs, was used according to

the following protocol.

First, we synthesized Cy3-labeled cDNA from 100 ng of total RNA using a one-color

Low Input Quick Amp Labeling kit (Agilent Technologies), according to the

manufacturer's instructions, followed by purification on an RNAeasy column (QIAGEN,

Valencia, CA, USA). Dye incorporation and cRNA yields were evaluated on a

NanoDrop ND-1000 spectrophotometer. Subsequently, 0.6 μg of Cy3-labelled cRNA

was fragmented at 60°C for 30 min in a reaction volume of 25 μL that contained 1x

Agilent fragmentation buffer and 2x Agilent blocking agent, according to the

manufacturer's instructions. On completion of the fragmentation reaction, 25 μL of 2x

Agilent hybridization buffer was added to the fragmentation mixture, which was

subsequently hybridized to the Agilent SurePrint G3 Human GE 8x60K Microarrays for

17 h at 65°C in a rotating Agilent hybridization oven. After hybridization, the

microarrays were washed for 1 min at room temperature with GE Wash Buffer 1

(Agilent) and for 1 min at 37°C with GE Wash buffer 2 (Agilent) and dried immediately

by brief centrifugation. The slides were scanned immediately after washing on an

Agilent DNA Microarray Scanner (G2565CA) using the one-color scan setting for

8x60k array slides (scan area: 61 mm $\times$ 21.6 mm, scan resolution: 3 μm, dye channel:

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100% Green PMT). The scanned images were analyzed using Feature Extraction

Software 10.10 (Agilent) with the default parameters to obtain the

background-subtracted and spatially detrended processed signal intensities.

Microarray data processing and analysis

The array data comprised 42,405 entities. The detection algorithm assigned a present,

marginal, or absent call to each entity according to the feature extraction. We used Gene

Spring GX software (Version 12.6; Agilent) to analyze the raw microarray data. Data

were initially selected using the present and marginal detection values, designated as

entities wherein at least 100.0% of samples in any one of the four groups (PM, DM,

CAM, and Control) had flags in the present or marginal categories. We then performed

a clustering analysis of all cases. The data were further filtered to determine fold

changes in expression relative to the Control group, and a GO analysis was performed

to filter the entities.

Real-time qRT-PCR

Real-time qRT-PCR was used to synthesize cDNA from 1 μg of total RNA using an

Oligo-dT primer (Promega, Madison, WI, USA). The primers for each candidate exon

were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/input.htm).

Real-time qRT-PCR was performed on an iCycler system (Bio-Rad Laboratories,

Hercules, CA, USA) with iQ CYBR Green Supermix (Bio-Rad). The reaction

conditions were 95°C for 3 min and 40 cycles of 10 s at 95°C (denaturation) followed

by 10 s at 55°C (annealing) and 30 s at 72°C(extension).

All experiments were performed in triplicate. The fluorescence emission spectra were

continuously monitored and analyzed with a sequence detection software. The expression of glyceraldehyde-3-phosphate dehydrogenase was simultaneously quantified as an internal standard control.

We identified eight genes related to the pathological condition from the GO results : *HLA-ABC*, *HLA-DR*, *HLA-DQ*, *CD8*, *ICAM-1*, *VCAM-1*, *MX-1*, and *AQP4*. These genes are all related to inflammatory pathology and muscle fiber damage and were used for the real-time qRT-PCR evaluation of the 14 initial cases. The expression ratio in each IIM group was calculated relative to the Control group.

The following primers were used:

HLA-ABC

5′-GGCTCTGATGTGTCCCTCA-3′ (forward)

5′-CAAGTCACAAAGGGAAGGGC-3′ (reverse)

HLA-DR

5′-CATGACAAAGCGCTCCAACT-3′ (forward)

5′-GTTGGGCTCTCTCAGTTCCA-3′ (reverse)

HLA-DQA1

5′-CCTCACCTTCCTCCCTTCTG-3′ (forward)

5′-GAATCTCAGGCTCCCAGTGT-3′ (reverse)

CD8

5′-GACGTGTTTGCAAATGTCCC-3′ (forward)

5′-TGTTGCACAGGGTTAGACGT-3′ (reverse)

ICAM-1

5′-GTGACCGTGAATGTGCTCTC-3′ (forward)

5′-CCTGCAGTGCCCATTATGAC-3′ (reverse)

VCAM-1

5′-GGGAAGATGGTCGTGATCCT-3′ (forward)

5′-GATTCTGGGGTGGTCTCGAT-3′ (reverse)

MX-1

5′-TCGGCAACAGACTCTTCCAT-3′ (forward),

5′-AAAGGGATGTGGCTGGAGAT-3′ (reverse)

AQP-4

5′-CTGGTCATGGTCTCCTGGTT-3′ (forward)

5′-TCAGTCCGTTTGGAATCACA-3′ (reverse).

Immunohistochemistry

Immunohistochemical procedures

For 62 cases, IHC analysis was performed using a standard avidin biotin-peroxidase complex method. The following primary antibodies were used: HLA-ABC (1:6000, mouse IgG1; Becton-Dickinson, San Jose, CA, USA), HLA-DR (1:300, mouse IgG1; Becton-Dickinson), HLA-DQ (1:300, mouse IgG1; Becton-Dickinson), CD8 (1:150, mouse IgG1; Dako, Glostrup, Denmark), ICAM-1 (1:50, mouse IgG1; Abd Serotec, Raleigh, NC, USA), VCAM-1 (1:50, mouse IgG1; Becton-Dickinson), MX-1 (1:1000, rabbit polyclonal; Gene Tex, Irvine, CA, USA), and AQP4 (1:200, rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Serial 6-μm thick sections were air dried and fixed in ice-cooled acetone for 3 min. The samples were then incubated with normal horse or goat sera (Vector Laboratories,

Burlingame, CA, USA) for 60 min, followed by incubation with the primary antibodies.

After washing with phosphate-buffered saline, the sections were incubated with

biotinylated horse anti-mouse IgG or biotinylated goat anti-rabbit IgG (Vector

Laboratories) for 60 min. Next, all sections were washed extensively, exposed to an

avidin-biotin complex (Vector Laboratories) for 60 min, and stained with

diaminobenzidine for 10 min.

Immunohistochemical analysis

   The expression of the protein product of each gene was analyzed semiquantitatively.

Overexpressed genes were evaluated on a scale of 0–4: 0 = no expression, 1 =

expression in 1%−25% of analyzed structures, 2 = expression in 26%−50%; 3 =

expression in 51%−74%, and 4 = expression in 75%−100%. Because *AQP4* expression

was decreased in IIM samples, the expression levels were analyzed as follows: 0 =

normal expression, −1 = expression in 75%−99% of analyzed structures, −2 =

expression in 51%−74%, −3 = expression in 26–50%, and −4 = expression in 0%−25%.

Statistical analysis

   The frequencies of each special protein product overexpressed or underexpressed

fiber were expressed as means ± one standard error. The three IIM groups were

compared using Scheffe's test. The real-time qRT-PCR results between pairs of groups

were compared using the *t*-test.

Results

In the clustering analysis of all 14 patients in a hierarchial dendrogram, the patients were divided into three groups: Control, PM, and DM + CAM. The PM group was distinct from patients in the original DM and CAM groups, whereas patients in the latter groups overlapped in the dendrogram (Figure 1).

Using a cut-off value of a twofold difference from the Control expression level, we created Venn diagrams of the genes that met this criterion (Figure 2). A total of 1609 genes were upregulated in all three groups. A GO analysis detected 385 ontologies ($p < 0.01$) reflective of a broad spectrum of inflammation, including immune system processes, humoral response, innate immune response, leukocyte activation, and cytokine mediated signaling pathway (Table 2). The 392 downregulated genes in all three groups were reflective of muscle fiber damage and homeostatic disorders.

Next, we performed the GO analysis to evaluate specific gene expression in each group. In the PM group, 1929 genes were specifically upregulated, corresponding to 45 ontologies ($p < 0.01$) reflective of cell-mediated immune responses such as leukocyte activation, cell adhesion, and regulation of T-cell activation. In the DM group, 205 genes were specifically upregulated and 365 were downregulated. However, ontologies were not detected by the GO analysis ($p < 0.05$). In the CAM group, 288 genes were specifically upregulated and 259 were downregulated; however, no ontologies were detected (Table 2).

We identified eight genes (*HLA-ABC, HLA-DR, HLA-DQ, CD8, ICAM-1, VCAM-1, MX-1, AQP4*) from the GO analysis and evaluated their expression levels via real-time qRT-PCR in the original 14 patient specimens. In the microarray analysis, *HLA-ABC* was equally expressed in all groups. *HLA-DR, HLA-DQ, CD8, ICAM-1,* and *VCAM-1*

were expressed more strongly in the PM group, whereas *MX-1* was expressed more strongly in the DM group. *AQP4*, which reflects muscle fiber damage, was strongly downregulated in the *PM* group (Table 3).

In the real-time qRT-PCR study, *HLA-DR, HLA-DQ, CD8, ICAM-1,* and *VCAM-1* were overexpressed most strongly in the PM group. *MX-1* was overexpressed in the DM and CAM groups but not in the PM group. *AQP4* expression was strongly downregulated in the PM group (Figure 3 A–H-1).

IHC analysis yielded similar results to those from the microarray and real-time qRT-PCR analyses (Figure 3 A–H-2, Figure 4). Major histocompatibility complex (MHC) class 1 and class 2 have an antigen-presenting function in the immune response. CD8 plays an important role in cell-mediated immunity. ICAM-1 and VCAM-1 are well known cell adhesion molecules involved in cell-to-cell interaction. MX-1 is an interferon α/β-inducible protein. AQP4 is a membrane protein belonging to a family of water channel proteins that facilitate water movement across biological membranes.

The expression of MHC class 1 (*HLA-ABC*) was high in all three groups. Notably, the expression levels of MHC class 2 (*HLA-DR, HLA-DQ*), CD8, and VCAM-1 were significantly higher in the PM group than those in the DM and CAM groups, and ICAM-1 expression was also higher in the former group. In contrast, the DM and CAM groups had significantly higher levels of MX-1 and AQP-4 expression ($p < 0.01$) than the PM group. Thus, the protein expression levels were similar to those detected via gene expression analysis. Notably, in the PM group, inflammation-related factors and cell adhesion molecules were much more strongly expressed than those in the DM and CAM groups, whereas there were many similar tendencies in expression and distribution between the latter two groups, making a distinction difficult.

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Discussion

We obtained similar results through a microarray, real-time qRT-PCR, and IHC analysis of specimens from patients with IIMs. We obtained three findings from this study: 1) DM and CAM are similar, 2) PM is different from DM and CAM, 3) the gene expressions of CAM are similar regardless of the type of cancer.

Many reports published in the last decade have described the application of microarray techniques in IIM research. Although some reports have analyzed PM and DM, few have investigated CAM. In these previous reports, microarray techniques were useful for the correct classification of muscle diseases such as IIMs, Duchenne muscular dystrophy, and nemaline myopathy (23). The expression of genes related to cytokines, MHC class 1 and 2 molecules, granzymes, adhesion molecules, and the actin cytoskeleton was found to be increased in cases of IIM. These genes are differentially expressed, depending on condition. Genes encoding cytokines, MHC class 1 and 2, and adhesion molecules are more strongly expressed in patients with PM and inclusion body myositis (IBM) than those in patients with DM. However, genes induced by interferon α/β, such as *ISG-15* and *MX-1*, are more strongly expressed in patients with DM than those in patients with PM and IBM (24).

In our study, we expanded the target of microarray analysis from PM and DM to CAM. We newly found that DM and CAM belong to the same group in a hierarchial dendrogram and have similar tendencies in the expression of pathology-related genes in GO analysis. On the other hand, PM was separated from other DM, CAM, and Control in the hierarchial dendrogram. Based on the results of a GO analysis, DM and CAM had no significant GO, but PM was associated with strong inflammation.

MHC class 1 expression on the sarcolemma, which is absent in normal muscle fibers, is upregulated in patients with IIMs (32-34). Additionally, MHC class 2 is not expressed on normal myofibers, and some authors have detected this antigen in specimens from patients with IIMs (35-38). HLA-DR was overexpressed on the sarcolemma in the PM group but weakly expressed in the DM and CAM groups in the present study. IHC staining with an HLA-DQ antibody revealed a similar pattern that confirmed the microarray data.

CD8 is overexpressed on inflammatory cells in patients with PM. In the present study, CD8 was significantly overexpressed on inflammatory cells in the PM group compared with the DM and CAM groups.

VCAM-1 and ICAM-1 are associated with IIMs, and a report described significantly upregulated ICAM and VCAM expression on blood vessels and muscle fibers in patients with IIMs relative to controls, which exhibited weak or absent expression (39-41). ICAM-1, and VCAM-1 expression was significantly stronger on sarcolemma and blood vessels in the PM group than that in the DM and CAM groups.

Greenberg et al. observed MX-1 via dense perifascicular staining and occasional staining of all myofibers and capillaries in patients with DM (24). In our study, MX-1 was overexpressed on some myofibers and capillaries, mainly perifascicular, in the DM group, whereas in some CAM group cases. Therefore, these results could suggest similarities between the DM and CAM groups.

AQP4　is expressed in healthy brain and muscle tissues (42) and well known to associate with neuromyelitis optica. AQP4 expression is also reduced in the skeletal muscle in patients with Duchenne muscular dystrophy, Fukuyama-type congenital muscular dystrophy, and sarcoglycanopathy (42-44). In the present study, IHC revealed

a decrease in AQP4 expression in both regenerating fibers and many non-necrotic fibers in patients with IIMs, particularly PM. This finding indicates that in IIMs, a decrease in AQP4 expression in the skeletal muscle causes a functional defect in skeletal muscle fibers that reflects a severe inflammation of the skeletal muscle.

Another report reviewed the muscle biopsy findings in patients with PM and DM with or without malignancy (31). They concluded that the incidence of rare-infiltrative type muscle pathology may be a predictive marker of DM with malignancy. In our IHC analysis of many cases, CAM was closely similar to DM regardless of cancer variation.

In addition, we found that MX-1 was strongly expressed in not only DM but also CAM, as discussed previously. In our study, the most common site-specific malignancy was lung cancer, and the most common pathological type was adenocarcinoma. Although various types of malignancies have been reported to associate with both DM and PM, the major types of malignancy vary depending on the study population and region. In a previous study conducted by Hill et al. in Sweden, Denmark, and Finland, the most common malignancy was ICD-7 code 162 or lung, trachea, and bronchus cancer (11). Other studies conducted in Western countries have reported adenocarcinoma of the ovary, lung, or gastrointestinal tract (10,15,26), whereas studies in Southeast Asia, Southern China, and Northern Africa report high rates of nasopharyngeal carcinoma (15,27-29). Regarding the pathological type of malignancy, another study concluded that adenocarcinomas, including lung, ovarian, cervical, gastric, and pancreatic cancers, were most commonly associated with DM (30). Our data confirmed the association of this classification using a gene expression profile.

Considering the results of this study, inflammation of PM was mainly mediated by the cellular immune system, and we believe that the muscle fiber is the main

inflammatory site in this condition. In contrast, DM and CAM did not show strong inflammation on muscle fibers and may thus exhibit similar humoral immune system-related pathologies. Additionally, these findings may indicate that a malignancy can induce muscle inflammation remotely and that the etiologies of CAM and DM share similar features. Our study may therefore provide a key to understanding the etiology of CAM and the clinical importance of cancer screening in patients with IIMs, particularly DM. Further studies are needed to confirm these findings.

The authors state that they have no Conflict of Interest (COI).

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Figure legends

Figure 1.

Cluster analysis of microarray data from polymyositis (PM; n = 3), dermatomyositis (DM; n = 3), cancer-associated myopathy (CAM; n = 3), and Control (n = 5) muscle specimens.

Hierarchical clustering was performed as described in the Patients and Methods section. The image presents all genes in rows and the 14 tissue samples in columns. Red, black, and green indicates high, intermediate, and low expression, respectively. We detected two initial tree branches that corresponded to normal specimens and inflammatory myopathies (IIMs; PM, DM, CAM). The IIM branch was further separated into two branches corresponding to PM and DM + CAM. This means DM and CAM are similar in gene expressions.

Figure 2.

Venn diagrams of the microarray results. The diagrams indicate the number of genes for which the expression levels in inflammatory myopathy samples differed from those in Control samples. Gene numbers were obtained by examining the list of genes with fold changes of at least 2. It means upregulated genes were overexpressed up more than double for Control, and downregulated genes showed decreased expression fewer than half for control. Many genes had a lot of ups and down in PM compared to DM and CAM.

  Abbreviations: CAM, cancer-associated myopathy; DM, dermatomyositis; PM, polymyositis

Figure 3.

Real-time qRT-PCR and immunohistochemistry (IHC) results.

A–H-1: Real-time qRT-PCR analysis results. Relative Expression indicates the gene expression in inflammatory myopathies (IIM) relative to the Control. *p < 0.05 vs. Control

A–H2: IHC staining grades. The following staining scale was used.

Overexpressed genes: 0 = no expression, 1 = expression in 1%–25% of the analyzed structures, 2 = expression in 26%–50%, 3 = expression in 51%–74%, and 4 = expression in 75%–100%. AQP4 expression was decreased in IIM and was graded according to the following scale: 0 = normal expression, −1 = expression in 75%–99%, −2 = expression in 51%–74%, −3 = expression in 26%–50%, and −4 = expression in 0%–25%. *p < 0.05; **p < 0.01.

Abbreviations: CAM, cancer-associated myopathy; DM, dermatomyositis; PM, polymyositis

Figure 4.

Photomicrographs of polymyositis (PM), dermatomyositis (DM), cancer-associated myositis (CAM), and Control specimens. Immunohistochemical reactions specific for HLA-ABC (A), HLA-DR (B), HLA-DQ (C), CD8 (D), ICAM-1 (E), VCAM-1 (F), MX-1 (G), and AQP4 (H) in PM, DM, CAM, and Control. Magnification, 200×.

Supplementary Figure

a) Patients enrolled in IHC study

b) Malignancies complicated in CAM patients (n=25)

Table 1. Clinical features of patients in the microarray and real-time qRT-PCR study

Pt No.	Age (years) /sex	Diagnosis	Disease Duration	Initial symptom	Rash	CK (IU/L)	Muscle biopsied /MRC Grade	Treatment for myopathy	Malignancy pathology/metastasis
1	62/M	PM	6M	Weakness	−	3329	Quadriceps/3	PSL	−
2	77/F	PM	6M	Weakness	−	312	Biceps/4	PSL + CyA	−
3	65/F	PM	3M	Weakness	−	10931	Biceps/4	PSL + AZ	−
4	62/F	DM	24M	Dysphasia	+	232	Biceps/4	PSL	−
5	35/M	DM	6M	Rash	+	863	Biceps/4	PSL	−
6	60/M	DM	5M	Rash	+	307	Biceps/4	PSL	−
7	59/M	CAM	1M	Rash	+	1101	Quadriceps/4	PSL	Larynx cancer SCC/−
8	55/F	CAM	8M	Weakness	−	1331	Biceps/4~3	PSL	Uterine corpus cancer Adeno/Lymph node
9	72/M	CAM	4M	Rash	+	700	Biceps/4	PSL	Prostate cancer Adeno/Bone, lung
10	57/M	Control	−		−	126	Biceps/5~4	−	−
11	70/F	Control	−		−	84	Quadriceps/4	−	−
12	17/M	Control	−		−	103	Quadriceps/5~4	−	−
13	21/M	Control	−		−	57	Biceps/4	−	−
14	36/M	Control	−		−	51	Biceps/5	−	−

PM: polymyositis, DM: dermatomyositis, CAM: cancer-associated myositis, CK: creatine kinase (normal value; 45～163 IU/L), MRC: Medical Research Council, PSL: predonisolone, CyA: cyclosporine, AZ: azathioprine, SCC: squamous cell carcinoma, Adeno: adenocarcinoma

Table2. Results of a gene ontology (GO) analysis based on the Venn diagrams

A. Genes with common variations among the three groups
- GO of 1609 upregulated genes
  - 385 GO ($p < 0.01$)
  - immune system processes, humoral response, innate immune response, leukocyte activation, cytokine mediated signaling pathway…, etc
- GO of 332 downregulated genes
  - 13 GO ($p < 0.05$)
  - muscle system processes, actin-myosin filament sliding, sarcomere, actin cytoskeleton…, etc

B. Genes with variations specifically in PM specimens
- GO of 1929 upregulated genes
  - 45 GO ($p < 0.01$)
  - immune response, leukocyte activation, cell adhesion…, etc
- GO of 951 downregulated genes
  - 79 GO ($p < 0.01$)
  - cellular respiration, mitochondrion, sarcomere… , etc

C. Genes with variations specifically in DM specimens
- GO of 205 upregulated genes
  - 0 GO ($p < 0.01$)
- GO of 356 downregulated genes
  - 0 GO ($p < 0.01$)

D. Genes with variations specifically in CAM specimens
- GO of 288 upregulated genes
  - 0 GO ($p < 0.01$)
- GO of 259 downregulated genes
  - 0 GO ($p < 0.01$)

PM, polymyositis; DM, dermatomyositis; CAM, cancer-associated myopathy

Table 3.
a) Fold changes (vs. Control) in the microarray analysis

Gene symbol	PM	DM	CAM
HLA-A	6.08±3.11	7.13±0.94	7.07±1.66
HLA-B	8.28±4.34	7.97±1.52	8.14±1.38
HLA-C	9.43±4.89	9.56±2.14	9.35±1.64
HLA-DRA	16.67±10.80	2.68±1.32	2.63±0.82
HLA-DRB1	10.18±6.30	2.68±1.32	2.15±0.47
HLA-DQA1	14.25±7.06	1.41±0.37	1.11±0.47
HLA-DQB1	29.69±25.70	5.63±3.77	1.83±1.09
CD8a	17.70±12.42	2.84±1.23	1.87±1.15
CD8b	16.28±13.46	4.88±2.22	3.06±2.10
ICAM-1	10.93±3.97	4.98±1.30	5.27± 1.86
VCAM-1	19.32±9.29	8.38±1.68	9.12±3.05
MX-1	8.75±0.54	58.04±14.12	48.19±39.27
AQP4	0.09±0.06	0.59±0.19	0.53±0.18

b) The expression of pathology-related genes is variable

	Gene symbol	
MHC Class I	HLA-ABC	PM = DM = CAM
MHC Class II	HLA-DR	PM >> DM = CAM
	HLA-DQ	PM >> DM $\geqq$ CAM
Lymphocyte/monocyte markers	CD8	PM >> DM = CAM
Adhesion molecules	ICAM-1	PM > DM = CAM
	VCAM-1	PM > DM = CAM
Interferon-induced protein	MX-1	PM << DM = CAM
Other	AQP4	PM << DM $\leqq$ CAM

PM, polymyositis; DM, dermatomyositis; CAM, cancer-associated myopathy

Figure 1.

Figure 2.

A. Upregulated

PM

DM

383

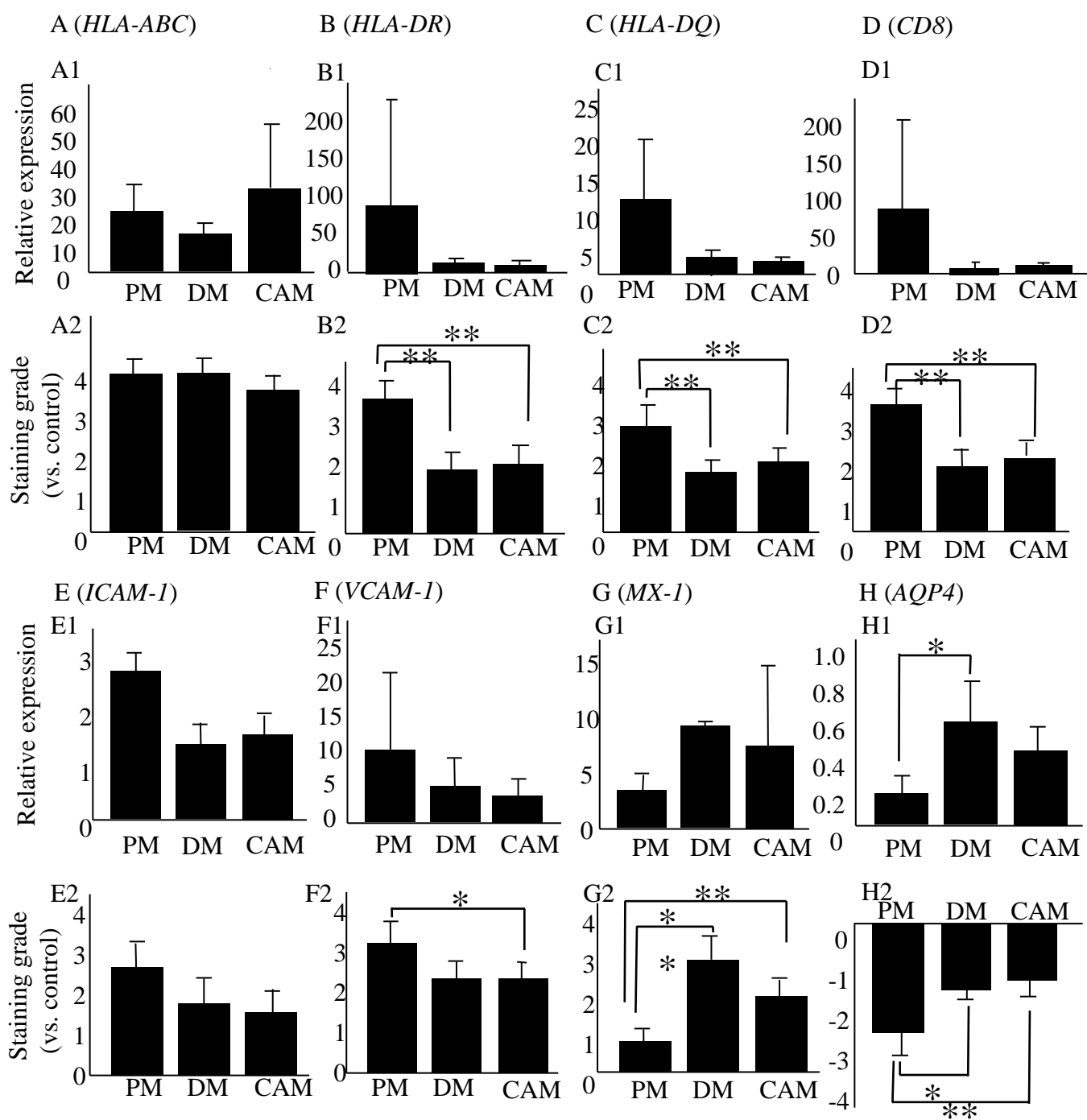205

1,929

1,609

142

288

501

CAM

B. Downregulated

PM

DM

951

140

356

332

219

106

259

CAM

A (*HLA-ABC*) B (*HLA-DR*) C (*HLA-DQ*) D (*CD8*)

E (*ICAM-1*) F (*VCAM-1*) G (*MX-1*) H (*AQP4*)

Figure 3.

	PM	DM	CAM	Control
A (HLA-ABC)				
B (HLA-DR)				
C (HLA-DQ)				
D (CD8)				
E (ICAM-1)				
F (VCAM-1)				
G (MX-1)				
H (AQP4)				

20 μm

Figure 4.

a) Subject of IHC study

	Number of patients（male/ female)	Mean age(y)
PM	14 (3/8)	61.6±12.8
DM	13 (2/8)	53.2±19.2
CAM	25 (11/11)	60.4±14.8
Control	10 (6/4)	44.2±18.2

b) Malignancies in CAM patients (n=25)

- lung cancer (n=6)
- gastric cancer (n=5)
- breast cancer (n=3)
- malignant lymphoma (n=3)
- tongue cancer (n=1)
- laryngeal cancer (n=1)
- esophageal cancer (n=1)
- hepatocellular carcinoma (n=1)
- bile duct cancer (n=1)
- uterine corpus cancer (n=1)
- ovarian cancer (n=1)
- prostate cancer (n=1)