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ORIGINAL ARTICLE

Serum-soluble TRAIL: a potential biomarker for disease activity in myositis patients

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Abstract

Objectives Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) is a member of the TNF super-family, which is involved in the regulation of immune response and pathogenesis of autoimmune diseases, including polymyositis (PM) and dermatomyositis (DM). In this study, we examined the level and origin of serum-soluble TRAIL (sTRAIL) in patients with PM and DM and analyzed its association with disease activity and clinical features.

Method 11 PM patients, 33 DM patients, and 20 healthy controls were enrolled in this study. Clinical features were recorded when admitted, and disease activity was evaluated by myositis disease activity assessment visual analogue scale (MYOACT). TRAIL expression in muscle tissues was detected by immunohistochemistry. Serum sTRAIL levels were measured by enzyme-linked immunosorbent assay. The expression of membrane TRAIL (mTRAIL) and its receptors, including DR4 and DR5, on circulating T cells was analyzed by flow cytometry.

Results TRAIL was expressed in infiltrated inflammatory cells in muscle tissues from patients. The serum sTRAIL level was markedly increased in patients and was positively correlated with the disease activity. Serum sTRAIL was decreased after therapy in patients and was specifically higher in patients with dysphagia, but lower in patients with autoantibody Jo-1 positive. The frequency of mTRAIL and its receptors on circulating T cells from patients were significantly elevated than that from healthy controls.

Conclusions The serum sTRAIL could be a biomarker for evaluating the disease activity of PM and DM, and targeting the generation of TRAIL in T cells might be a potential approach in the treatment of PM and DM.

Keywords Biomarker · Dermatomyositis · Polymyositis · Serum TRAIL

Introduction

Polymyositis (PM) and dermatomyositis (DM) are systemic autoimmune diseases characterized by chronic muscle weakness and inflammation, leading to significant morbidity and mortality [1, 2]. The infiltration of imflammatory cells in the mussle tissue is the primary pathological features in these diseases. Endomysial infiltrates in PM primarily includes CD8+ T cells, CD4+ T cells, DCs, and macrophages, while perivascular infiltrates in DM are mainly composed of CD4+ T cells, DCs, macrophages, and B cells [3, 4]. Targeting these infiltrates, especially T cells, may be a promising future approach in the treatment of PM and DM [5], but rigorous study of the role of T cells in these diseases still needs to be performed.

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), also known as Apo2 ligand, is a member of the TNF super-family, which has a pro-apoptotic activity in cells expressing at least one of their specific death receptors, DR4 (TRAIL-R1) or DR5 (TRAIL-R2) [6–8]. Two forms of TRAIL are existed in the body, that is, membrane-bound TRAIL (mTRAIL) and soluble TRAIL (sTRAIL). TRAIL is considered to be involved in the regulation of immune response and pathogenesis of autoimmune diseases [9–11]. It is reported that TRAIL is upregulated in human myositis muscle tissues and mediates muscle fiber damage via autophagy in

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human and animal myositis [12, 13]. Considering that TRAIL mainly exerts its role through its soluble form [14], it is necessary to detect the levle of serum sTRAIL and analyze its role in these diseases.

In this study, we measured serum concentrations of sTRAIL in patients with PM and DM and assessed the relationship between the TRAIL levels and clinical features in PM and DM patients. In addition, we also observed the expression of mTRAIL and its receptors on circulating T cells.

Materials and methods

Patients and samples

Peripheral blood, serum, and muscle samples were collected from 44 patients with PM or DM who were admitted to the Beijing Friendship Hospital between 2016 and 2018. PM and DM were diagnosed according to the criteria developed by Bohan and Peter [15, 16]. In addition, the myositis disease activity assessment visual analogue scale (MYOACT), established by the International Myositis Assessment and Clinical Studies (IMACS) group, was used to evaluate the disease activity [17]. This tool was proved to be reliabe for evaluating disease activity in Chinese patients with PM and DM in our previous study [18]. All patients were evaluated before treatment and subsequently followed up for 3 to 6 months. Disease control group included 20 healthy volunteers matched for age and sex to the patients. Patients with clinically amyopathic DM (CADM) is not included in this study. The characteristics of all subjects in this study are summarized in Table 1.

Patients were excluded if they had a history or evidence of any autoimmune diseases other than PM and DM, evidence of a serious uncontrolled disease, history or evidence of tuberculosis, active infections, history of hereditary or acquired immune deficiency disorder, and were pregnant or breast-feeding. Interstitial lung disease (ILD) was diagnosed by clinical findings as follows: pulmonary complaints, lung auscultation, pulmonary function testing, and reticular shadow on chest radiographs or ground-glass opacity on chest high-resolution computed tomography.

Immunohistochemistry

Muscle specimens from all patients were obtained by muscle biopsy from deltoid or quadriceps femoris. Immunohistochemistry was performed on formaldehyde-fixed, paraffin-embedded muscle specimens. After hydration, muscle sections were incubated with anti-TRAIL antibody (CST, Cat. 3219) for 1 hour at 37 °C, and HRP conjugated secondary antibody (Abcam) was used to display the signals.

Table 1 Characteristic of patients

Characteristic	PM ($n = 11$)	DM ($n = 33$)	Healthy controls ($n = 20$)
Male/female	4/7	5/28	3/17
Age mean (range)	40 (24–63)	43 (18–63)	41 (18–60)
Muscle tenderness	4 (36.4%)	10 (30.3%)	
Muscle weakness	8 (72.7%)	13 (39.4%)	
Heliotrope	0 (0.0%)	20 (60.6%)	
Gottron sign	0 (0.0%)	18 (54.5%)	
V sign	0 (0.0%)	11 (33.3%)	
Mechanic's hand	0 (0.0%)	11 (33.3%)	
Raynaud's phenomenon	1 (9.1%)	4 (12.1%)	
Arthralgia	4 (36.4%)	10 (30.3%)	
Dysphagia	2 (18.2%)	5 (15.1%)	
ILD	2 (18.2%)	13 (29.4%)	
ANA	5 (45.5%)	11 (33.3%)	
SSA	3 (27.2%)	8 (24.2%)	
RNP	2 (18.2%)	2 (6.1%)	
Jo-1	2 (18.2%)	3 (9.1%)	

ILD interstitial lung disease, *ANA* antinuclear antibody, *SSA*, anti-SSA/Ro antibody, *RNP* antiribonuclear protein antibody

Blood examination

The routine blood examinations included complete blood count, serum chemistries, urinalysis, and autoantibodies. All biochemical parameters were detected by standard automated laboratory methods using commercially available kits according to the manufacturers protocols. sTRAIL concentrations in the sera were measured by a commercial ELISA kit (MyBiosource, USA) according to the manufacturer's instruction. All serum samples were stored at − 80 °C and then analyzed on the same day.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation using Ficoll-Hypaque (Sigma). Cells were surface stained with anti-CD3-PE (Miltenyi Biotec, Cat. 130-019-374) and anti-TRAIL-FITC (Miltenyi Biotec, Cat. 130-117-559), or anti-DR4-FITC (Miltenyi Biotec, Cat. 130-109-034), or anti-DR5-FITC (Miltenyi Biotec, Cat. 130-102-478). Cell were analyzed using a FACSCalibur (BD) flow cytometry. T cells are defined as CD3+. Data were analyzed in Flowjo.

Statistical analysis

All analyses were carried out with the SPSS 19.0 software (Chicago, IL, USA). Data were shown as mean ± SD. The significance of difference between two groups was identified

using a Student's *t* test. Multiple comparisons were performed by one-way ANOVA and followed by Bonferroni post test for comparison between two groups. Spearman's correlation analysis was used to test for correlations. *P* values less than 0.05 were considered significant.

Results

Expressions of mTRAIL in muscle tissues from PM and DM patients

This study enrolled 44 patients (11 PM and 33 DM) and 20 health controls, and the characteristics of all subjects are summarized in Table 1. The result of HE staining showed that musle fibers were damaged and numerous mononuclear cells were infiltrated in the musle tissue from patients PM and DM (Fig. 1a). The expression of mTRAIL in the affected muscle tissues in patients with PM and DM was examined by immunohistochemistry. The signals of mTRAIL were weak in control samples, while the signals of mTRAIL were strong in the muscle tissues of those with PM and DM, and the signals were primarily located in the infiltrated mononuclear cells (Fig. 1b).

Serum levels of sTRAIL in patients with PM and DM

We detected the concentrations of serum sTRAIL in patients with PM and DM, as well as in healthy controls. There was no obvious difference between the level of serum sTRAIL in PM patients (1403.72 ± 397.04 ng/L) and that in DM patients (1371.30 ± 194.14 ng/L) (Fig. 2a). However, serum sTRAIL in both PM and DM patients was markedly increased compared with that in healthy controls (493.89 ± 33.32 ng/L) (Fig. 2a). Importantly, a positive correlation was observed between serum level of sTRAIL and disease activity scoring by MYOACT tool ($r = 0.833$, $P < 0.001$) (Fig. 2b). In addition, we found that serum level of sTRAIL was significantly decreased in patients with PM and DM after 3 to 6 months of therapy, especially in patients whose serum sTRAIL level was at a high level before therapy (Fig. 2c).

Serum levels of sTRAIL in patients with different clinical features

We investigated the association between serum level of sTRAIL and clinical features in PM and DM patients. First, we analyzed the difference of serum levels of sTRAIL between patients with and without a certain symptom, including muscle tenderness, skeletal muscle weakness, Heliotrope, Gottron sign, V sign, mechanics' hand, Raynaud's phenomenon, arthralgia, dysphagia, and ILD (Table 2). We found that serum levels of sTRAIL in patients with dysphagia (1918.6 ± 324.95 ng/L) were significantly higher than that in patients without dysphagia (886.3 ± 112.66 ng/L) ($P = 0.014$) (Table 2). Second, we examined the difference of serum levels of sTRAIL between patients with and without elevation of an autoantibody, such as ANA, SSA, RNP, and Jo-1. Our data showed that serum levels of sTRAIL in Jo-1 positive group (562.36 ± 52.99 ng/L) were obviously lower than that in Jo-1 negative group (1334.57 ± 181.21 ng/L) ($P = 0.03$) (Table 2). Finaly, correlation analysis showed that serum levels of sTRAIL were not correlated with muscle enzymes, such as creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) (Fig. 3).

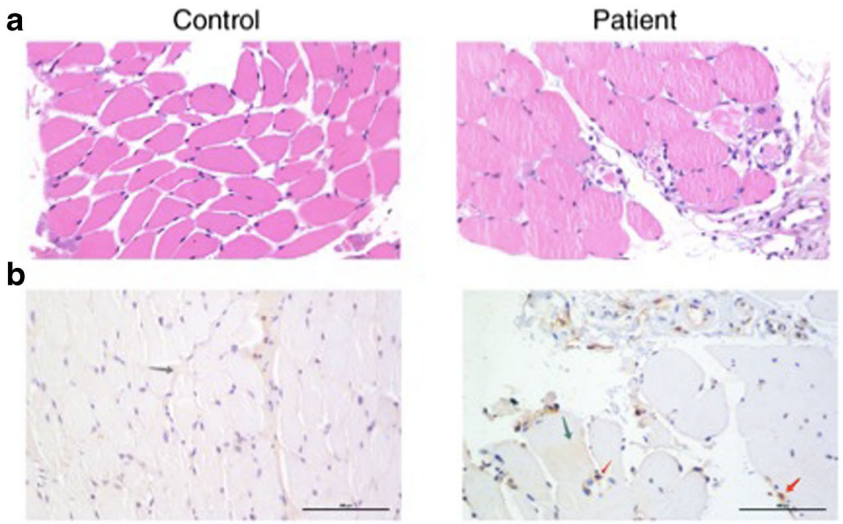

Fig. 1 Representative sections showing HE and immunohistochemistry staining of muscle tissues. **a** HE staining of muscle tissues from control group and PM/DM patients. **b** Expression of TRAIL in muscle tissues from control group and PM/DM patients detected by immunohistochemistry. Gray arrow indicates the expression of TRAIL in myocyte, and red arrow indicates the expression of TRAIL in lymphocyte. Scale bar, 100 μm

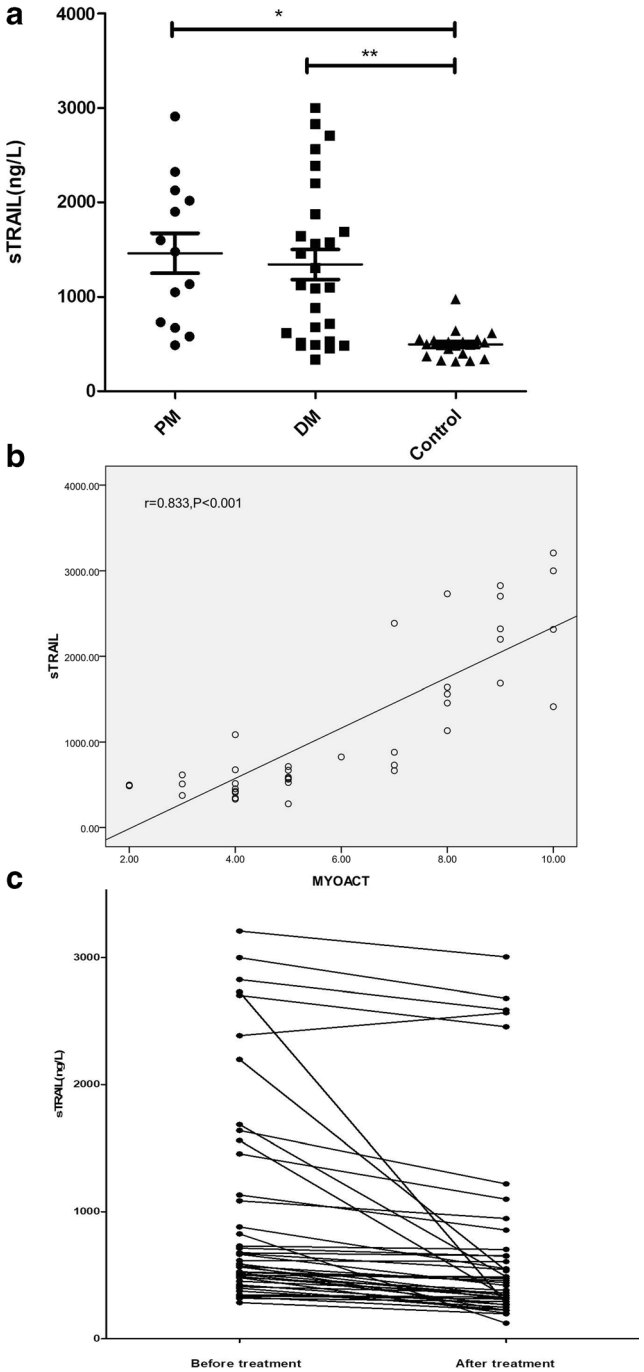

Fig. 2 Serum sTRAIL levels in patients and healthy controls. Serum samples were collected from PM patients ($n = 11$), DM patients ($n = 33$), and healthy controls ($n = 20$). **a** Concentration of serum sTRAIL measured by ELISA. **b** Correlation analysis betwenn serum sTRAIL level and disease activity. **c** Serum sTRAIL levels before and after treatment in PM and DM patients. Symbols joined by a solid line represent data from an individual subject

Expression of mTRAIL and its receptors on circulating T cells

We analyzed the expression of mTRAIL and its receptors on circulating T cells from patients with PM and DM patients, as well as from healthy controls, by flow cytometry. Our data showed that the frequency of mTRAIL and its receptors, including DR4 and DR5, on circulating T cells from PM and DM patients was significantly elevated than that from healthy controls (Fig. 4a–c). In addition, we found that the frequency of mTRAIL on circulating T cells was obviously correlated with the serum level of sTRAIL (Fig. 4d).

Discussion

In this study, we found that TRAIL was expressed in infiltrated inflammatory cells in muscle tissues from PM and DM patients. The serum sTRAIL was markedly increased in patients with PM and DM and was correlated with the disease activity. Serum sTRAIL was specifically higher in patients with dysphagia, but lower in patients with Jo-1 positive. The frequency of mTRAIL and its receptors, including DR4 and DR5, in circulating T cells from PM and DM patients was significantly elevated than that from healthy controls.

The levels of serum sTRAIL obtained from patients with PM and DM were significantly elevated, and the serum sTRAIL levels were markedly decreased after therapy, especially in patients whose serum sTRAIL level were at a high level before therapy. Importantly, the serum sTRAIL levels were obviously associated with the disease activity of myositis, scoring by MYOACT tool. Therefore, serum sTRAIL could be a biomarker for evaluating the disease activity of PM and DM. Importantly, serum sTRAIL levels were also elevated in other rheumatic diseases, such as systemic lupus erythematosus [19], scleroderma [10], and early rheumatoid arthritis [20], which implied the serum sTRAIL might be a common indicator for a series of autoimmune diseases.

In addition, we found that serum levels of sTRAIL in patients with dysphagia were distinctly higher than that in patients without dysphagia, which implied that patients with high level of serum sTRAIL were susceptible to dysphagia. We also found that serum levels of sTRAIL in Jo-1 positive group were obviously lower than that in Jo-1 negative group. Unfortunately, our present data still could not account for the mechannism behind these two phenomena. It is reported that TRAIL mediates muscle fiber damage in human and animal myositis. Dysphagia is a symptom resulted from the damage of esophageal smooth muscle cells. Therefore, we speculated that TRAIL was directly involved in the pathogenesis of dysphagia. On the other hand, the expression of myositis autoantigen, such as Jo-1, is primarily increased in regenerating muscle cells [21], and patients with non-Jo-1 autoantibodies have worse survival than Jo-1 positive patients [22]. Thus, Jo-1 positive might reflect the activity of regeneration of muscle cell in patients, whose serum sTRAIL is lower.

Table 2 The concentration of serum sTRAIL in groups with different symptoms

Symptoms	With	Without	*P* value
Muscle tenderness	1086.83 ± 219.89	1471.98 ± 263.30	0.156
Muscle weakness	1117.28 ± 182.13	1684.73 ± 378.22	0.283
Heliotrope	1386.60 ± 220.84	1011.43 ± 235.98	0.088
Gottron sign	1299.54 ± 204.07	1243.17 ± 328.17	0.36
V sign	1221.39 ± 248.90	1322.92 ± 243.31	0.536
Mechanic's hand	952.98 ± 141.31	1490.63 ± 259.59	0.06
Raynaud's phenomenon	1305.37 ± 422.48	1056.37 ± 157.04	0.81
Arthralgia	1181.99 ± 239.99	1376.82 ± 253.63	0.745
Dysphagia	1918.6 ± 324.95	886.3 ± 112.66	0.014
ILD	1309.40 ± 232.21	1244.80 ± 266.79	0.964
ANA	1084.87 ± 146.91	773.91 ± 228.11	0.10
SSA	1123.74 ± 289.72	1380.13 ± 893.86	0.99
RNP	1317.10 ± 506.50	1005.19 ± 133.15	0.05
Jo-1	562.36 ± 52.99	1334.57 ± 181.21	0.03

ILD interstitial lung disease, *ANA* antinuclear antibody, *SSA*, anti-SSA/Ro antibody, *RNP* antiribonuclear protein antibody

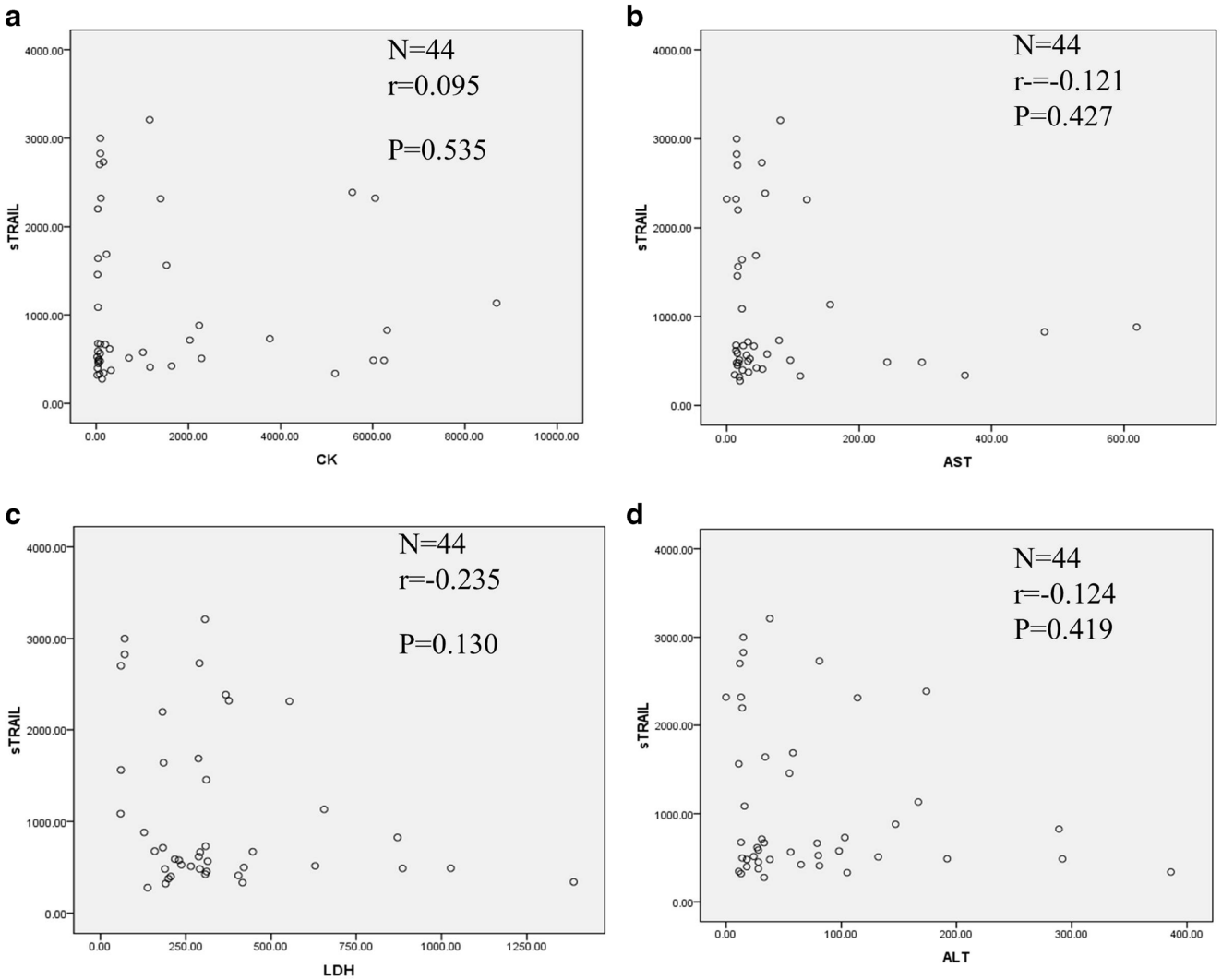

Fig. 3 Correlation analysis betwenn serum sTRAIL level and muscle enzymes

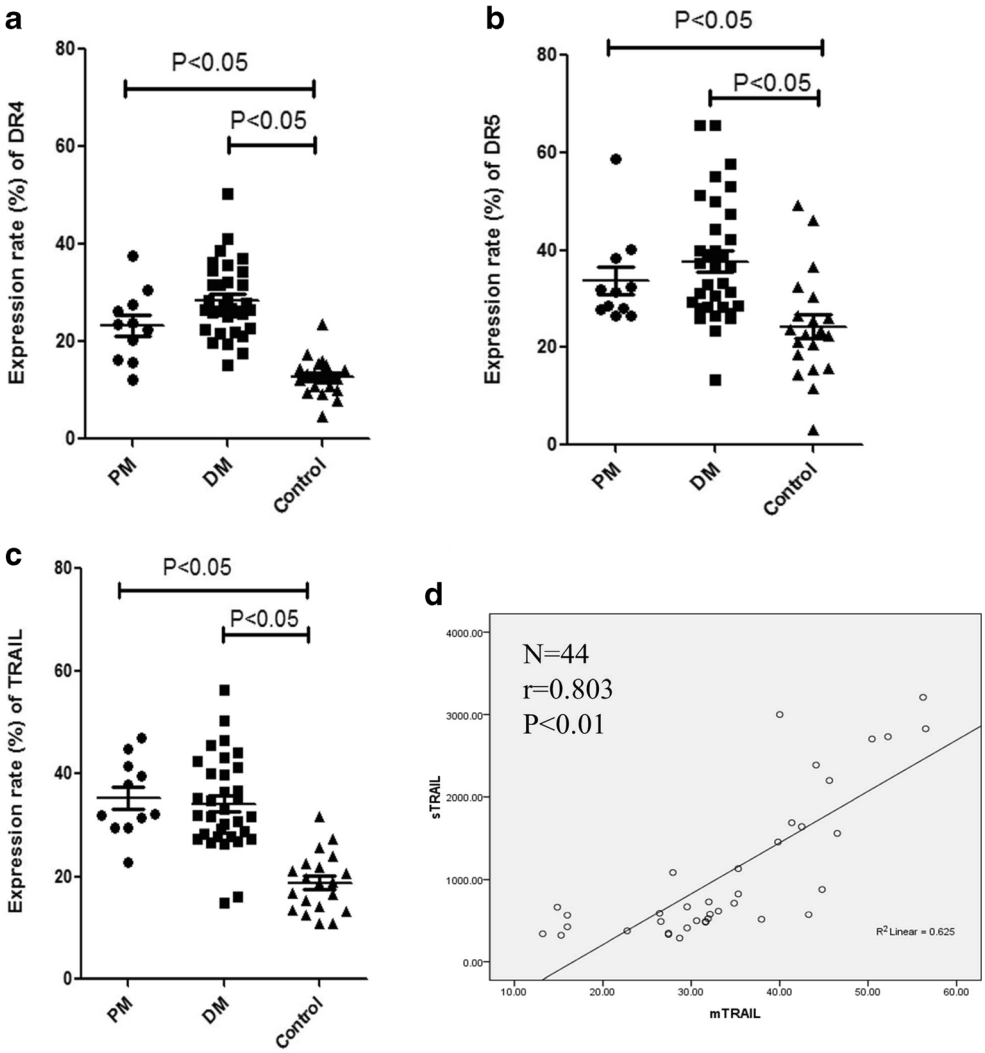

Fig. 4 Expression of TRAIL and its receptors on peripheral blood T cells. Peripheral blood samples were collected from PM patients ($n = 11$), DM patients ($n = 33$), and healthy controls ($n = 20$). **a**–**c** Expression of TRAIL (**a**), DR4 (**b**), and DR5 (**c**) on circulating T cells analyzed by flow cytometry. **d** Correlation analysis between the frequency of mTRAIL on circulating T cells and serum level of sTRAIL

It is well documented that the muscle dysfunction and weakness in PM and DM is the result of muscle tissue damage, but the molecular mechanisms behind it remain poorly understood [1]. TRAIL has been previously reported to be upregulated in biopsy specimens from myositis patients and mediate muscle tissue damage via autophagy in human and animal myositis [12]. In this study, our data also showed that TRAIL expression was elevated in the muscle tissues from PM and DM patients. Importantly, we showed that their serum sTRAIL levels were also increased, which suggested that TRAIL could exert its role locally and systemically. In addition, our data indicated that circulating T cells were a primary source of serum sTRAIL, because the frequency of mTRAIL in circulating T cells was obviously correlated with serum level of sTRAIL. Cytotoxic T cells have been reported to one of the main infiltrated cells invaded the muscle fibers in PM and DM patients [3]. Thus, the elevation of TRAIL in muscle tissues might derive from the infiltration of cytotoxic T cells, and cytotoxic T cells could induce the muscle cell death through membrane and soluble TRAIL. It is intriguing that cirulating T cells also expressed high level of the death receptors of TRAIL, including DR4 and DR5. Previous study implied that, in addition to inducing apoptosis by binding to the death receptors, TRAIL can enhance T cell proliferation after TCR engagement [23]. We proposed that T cell-derived TRAIL might have dual role in the pathogenesis of myositis: it induced the muscle cell death but promoted the proliferation of T cells. Therefore, targeting the generation of TRAIL in T cells might be a potential approach in the treatment of PM and DM. However, future experiments using TRAIL-knockout mice or pharmacologic anti-TRAIL interventions are needed to clarify the role of TRAIL in the pathogenesis of myositis.

In conclusion, our study highlighted that TRAIL might play an important role in the pathogenesis of PM and DM. The serum sTRAIL could be a biomarker for evaluating the disease activity of PM and DM, and targeting the generation of TRAIL in T cells might be a potential approach in the treatment of PM and DM.

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Compliance with ethical standards

The study was approved by the Ethics Committee of Beijing Friendship Hospital (approved number: 2018-P2-204-01). Informed consent was obtained from all enrolled participants for their data to be used for this study. Patients did not receive any financial compensation.

Disclosures None.

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