RESEARCH ARTICLE

WILEY

Activation of cGAS-STING pathway – A possible cause of myofiber atrophy/necrosis in dermatomyositis and immune-mediated necrotizing myopathy

Meichen Zhou[1] | **Xiaoxiao Cheng[1]** | **Wenhua Zhu[2]** | **Jianhua Jiang[1]** | **Sijia Zhu[1]** | **Xuan Wu[3]** | **Meirong Liu[1]** | **Qi Fang[1]**

[1]Department of Neurology, First Affiliated Hospital of Soochow University, Suzhou, China

[2]Department of Neurology, Huashan hospital, Shanghai, China

[3]Department of Neurology, Affiliated Hospital of Yangzhou University, Yangzhou, China

Correspondence
Meirong Liu and Qi Fang, epartment of Neurology, first affiliated hospital of Soochow University, Suzhou, China.
Emails: meizai26@163.com and fangqi_008@126.com

Abstract

Objective: The objective was to investigate the expression of the cGAS-STING pathway-associated protein in idiopathic inflammatory myopathy (IIM) and to investigate whether it is related to myofiber atrophy/necrosis in patients with dermatomyositis and immune-mediated necrotizing myopathy.

Material and Methods: Muscle specimens obtained by open biopsy from 26 IIM patients (14 with dermatomyositis (DM), 8 with immune-mediated necrotizing myopathy (IMNM), and 4 with other types of IIM), 4 dystrophinopathy, and 9 control patients were assessed for expression of cGAS-STING pathway members via Western blot, quantitative real-time PCR analysis (qRT-PCR), and immunochemistry. Meanwhile, analysis its location distribution throug immunochemistry.

Results: Compared to the control group, the expression of cGAS, STING, and related molecules was obviously increased in muscle samples of IIM patients. Upregulated cGAS and STING were mainly located in the vascular structure, inflammatory infiltrates, and atrophic and necrotic fibers. While comparing to the Dys patients, the mRNA level of cGAS, STING, and TNF-a was upregulated, meanwhile, the protein of the TBK1, P-TBK1, and P-IRF3 associated with interferon upregulation was overexpressed through Western blot in IMNM and DM. Considering that cGAS and STING are located in necrotic and Mx1-positive atrophic fibers, it is really possible that the cGAS-STING pathway may lead to fibers atrophy/necrosis by producing IFNs.

Conclusion: The cGAS-STING pathway was activated in the muscle samples of IIM patients and its activation may be the reason of myofiber atrophy and necrosis in DM and IMNM patients.

KEYWORDS
cGAS-STING pathway, dermatomyositis, immune-mediated necrotizing myopathy

---

Meichen Zhou, Xiaoxiao Cheng and Wenhua Zhu contributed equally to this work.

1 | INTRODUCTION

Idiopathic inflammatory myopathy (IIM) is a heterogeneous family of chronic systemic autoimmune diseases, which include dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), antisynthetase syndrome (AS), and sporadic inclusion body myositis (sIBM).[1] The general pathological manifestations are muscle inflammation, muscle atrophy, muscle necrosis, and regeneration. Although the exact cause of IIM remains unclear, the interferons (IFNs) have been suggested to have a critical role in its pathogenesis and progression.[2–5] IFNs can be classified into three types based on their specific receptors: type I IFN (IFN-I), type II IFN (IFN-II), and type III IFN (IFN-III). Several studies have confirmed the presence of IFN signature, especially IFN-I, in the muscle,[6] peripheral blood,[7,8] and skin[9,10] of patients with DM.[11] Also, human myxovirus resistance protein 1 (MX1), a type I interferon-inducible protein, has been considered as a sensitive diagnostic marker of DM.[12] Moreover, recent studies have found that IFNI-signaling inhibitor ruxolitinib treatment improves refractory dermatomyositis facial skin rash and the quality of patient's life.[13,14] In addition, RNA sequencing data show that the IFN pathway is activated not only in patients with DM, but also in patients with ASS, IMNM, and sIBM.[3] IFN-r, one cytokine of IFN-II, messenger is significantly higher in IMNM than control.[15] However, the main source of IFNs in IIM remains unclear.

Cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING), is primarily involved in response to microbial infection. In addition, recent studies have revealed its fundamental role in developing a variety of inflammatory diseases and autoimmune diseases.[16] Constitutive cGAS-STING pathway activation and associated effector functions, such as interferon production, can result in cell or tissue damage. Consistent with this notion, dysregulation of the cGAS–STING signaling pathway in adipocytes,[17] hepatocytes,[18] and renal tubule cells[19–21] are associated with metabolic dysfunction, impaired energy homeostasis, and kidney diseases. Beyond the canonical role of cGAS-STING in mediating cytokine production, growing evidence highlights the emerging role in regulating autophagy and cell death (e.g., apoptosis, necroptosis, pyroptosis, ferroptosis, mitotic cell death, and immunogenic cell death).[22,23] As in MEF cells, STING is a potential autophagy receptor and directly interacts with LC3 via its LIR motifs to mediate autophagy and its own autophagic degradation to tune the innate immune response.[24]

Mechanistically, cGAS can sense the presence of cytosolic DNA, after which cGAS is activated to generate the second messenger cGAMP, in turn activating STING in the endoplasmic reticulum. STING is then transported from the ER to the Golgi, where it forms a complex that, with TANK-binding kinase 1 (TBK1), is then transferred to the endolysosome, where it stimulates transcription factors, such as interferon regulatory factor 3 (IRF3) and nuclear factor-kB (NF-kB). These transcription factors facilitate the expression of interferons or pro-inflammatory cytokines, such as IFNs and tumor necrosis factor (TNF). Moreover, emerging evidence showed that the cGAS-STING pathway is not only activated by non-self DNA, such as DNA from viruses, bacteria, and protozoa, but also activated by self-DNA[25,26] including intracellular, mitochondrial, and nuclear DNA[27,28] which can enter the cytoplasm.

Based on the role of cGAS-STING in mediating cytokine production and in regulating autophagy and cell death, we hypothesized that the cGAS-STING pathway might be involved in DM and IMNM pathogenesis. In our study, we compare the expression of the cGAS-STING pathway-associated protein and investigate possible role in patients with DM and IMNM.

2 | MATERIAL AND METHODS

2.1 | Patients

Muscle specimens were retrospectively obtained from 26 patients diagnosed with IIM according to European Neuromuscular Center criteria.[29] Among the 26 patients with IIM, 14 had dermatomyositis (DM), 8 had immune-mediated necrotizing myopathy (IMNM), and 4 had non-specific IIM (NSIIM or NSM) which have muscle inflammation with insufficient biopsy criteria to allow subclassification. Among them, five muscle biopsies were from Huashan Hospital. Four patients were diagnosed according to their clinical features, and pathological and genetic results were included in the dystrophinopathy group (FSHD = 1 LAMA2 = 1, LGMD2H = 1, and BMD = 1). In addition, nine controls initially suspected of having muscle disorders were included in the study. Open biopsies were performed for all patients. Normal histological findings were found, and these non-disease controls did not have any neuromuscular disease with longer follow-up times. The profiles of IIM patients are summarized in Table 1.

No other neurologic or immunologic diseases were present. No information on the history of viral infections was available for any patient or control. No patient received immunosuppressive drugs before the biopsy.

The Institutional Review Board of the first affiliated hospital of Soochow University approved the study. All patients signed the informed consent.

2.2 | Western blot

Western blotting was performed on muscle homogenates. A BCA assay kit (ES science Ltd, ES6002) was used to determine protein concentration. Briefly, 10ug of protein was loaded into 7.5%–12.5% SDS-polyacrylamide gel for electrophoresis. Samples were then transferred onto polyvinylidene difluoride (PVDF) membranes and incubated with primary antibodies including anti-cGAS (Abcam, Cat# ab224144, RRID: AB_2904576), anti-STING (Cell Signaling Technology, Cat# 13647, RRID: AB_2732796), anti-p-STING (Cell Signaling Technology, Cat# 50907, RRID: AB_2827656), anti-p-IRF3 (Cell Signaling Technology, Cat# 29047, RRID: AB_2773013), anti-TBK1 (Proteintech, Cat# 28397-1-AP, RRID:AB_2881132), anti-p-TBK1(Signalway Antibody, Cat# 13488, RRID: AB_2904577),

TABLE 1 Clinical and pathological profile in IIM patients

Patients no.	Sex (F:M)	Age	Disease duration (Mon)	MSA	Skin rash	PFA	Inflammatory infiltrates	Necrosis
1	F	64	8	PM-Scl100	Y	Y	++	+
2	M	80	12	TIFr	Y	Y	++	+
3	M	24	5	MDA5	Y	Y	++	–
4	F	24	12	NXP2	Y	Y	++	–
5	F	48	36	TIF1r , PL-7	Y	Y	+	–
6	F	42	3	NXP2	N	Y	++	+
7	F	52	12	TIF1	Y	Y	++	–
8	M	54	1	NXP2	Y	N	++	–
9	M	57	0.25	–	Y	N	+	–
10	F	51	2	MDA5	Y	N	++	+
11	F	81	2	TIF1	Y	Y	++	+
12	M	52	1	MDA5	Y	N	++	+
13	F	74	2	–	Y	N	+	–
14	F	65	1	–	Y	N	+	–
15	F	27	24	HMGCR	N	N	++	+++
16	F	28	48	SRP	N	N	++	+++
17	M	50	6	SRP	N	N	+	+++
18	F	72	36	–	N	N	+	++
19	F	67	24	NA	N	N	+	++
20	F	83	2	OJ	N	N	++	++
21	F	56	8	NA	N	N	++	+++
22	F	26	3	NA	N	N	++	+++
23	F	62	5	–	N	N	–	–
24	M	54	36	–	N	N	++	–
25	M	30	2	Mi-2	N	N	++	+
26	M	49	2	–	N	N	+	–

Abbreviations: –, negative; +, mild; ++, moderate; +++, severe; MSA, myositis-specific antibody; N, no; Y, yes.

anti-p-NFkB (Cell Signaling Technology, Cat# 3033, RRID: AB_331284), and GAPDH (Cell Signaling Technology Cat# 5174, RRID: AB_10622025). Finally, chemiluminescence was detected by Western blot luminol reagent, and proteins were quantified using Image J software.

2.3 | Quantitative real-time PCR analysis (qRT-PCR)

Myotubes were processed, and RNA was extracted using the tissue RNA purification kit plus (ES science, Ltd., ES-RN002plus) following the manufacturer's instructions. RNA concentration was determined, and cDNAs were synthesized from 0.2 μg of total RNA using a reverse transcription kit (ES science, Ltd., ES-RT001). The synthesized cDNAs were used as templates for qRT-PCR performed with the 7500 FAST Real-Time PCR system (Roche) using SYBR Green Master Mix (Thermo Fischer, A25742) in a 20-μl volume. The following primers were used: GAPDH: 5′-CATGTTCGTCATGGGTGTGAACCA-3′ (Forward),

5′-AGTGATGGCATGGACTGTGGTCAT-3′ (Reverse); cGAS: 5′-AGA AGGCCTGCGCATTCAAA-3′ (Forward), 5′-GCCGCCATGTTTCTTC TTGGA-3′ (Reverse); STING: 5′-GAAATTATTCCTGCAAGCCAAT TT-3′ (Forward), 5′-TCACCCTTCTTTTTCATGTAGCA-3′ (Reverse); TNF-a: 5′-TCTCTCAGCTCCACGCCATT-3′ (Forward), 5′-CCCAGGC AGTCAGATCATCTTC-3′ (Reverse). The change in the relative expression of each gene was calculated using the comparative CT method ($2^{-\triangle\triangle CT}$).

2.4 | Immunohistochemistry

Eight-micrometer cryostat sections of muscle tissue were air-dried at room temperature for 30 min and then fixed in cool acetone (4°C) for 10 min. The PV-9000 polymer detection system (ZsBio Ltd.) was used. After rehydrating in phosphate-buffered saline (PBS), all sections were incubated overnight at 4°C, with the following primary antibodies (brand, number, and dilution): anti-cGAS (Proteintech, Cat#26416, RRID: AB_2880507), anti-STING

(Proteintech, Cat# 19851-1-AP, RRID: AB_10665370), anti-MX1 (Abcam, Cat# ab95926, RRID: AB_10677452), and anti-CD68 (ZSGB-Bio , Cat# ZM-0060, RRID: AB_2904190). Then, sections were incubated with polymer helper for 30 min, followed by polymerized horseradish peroxidase-conjugated anti-mouse/rabbit immunoglobulin G (IgG) for 30 min at 37°C. Peroxidase activity was determined using diaminobenzidine. Finally, slides were counterstained with hematoxylin and eosin, dehydrated, and mounted using a neutral balsam. The quantification of immunohistochemical staining intensities was performed using the Image J software. We captured at least five fields from perifascicular regions of DM, necrosis fibers of IMNM, and random fibers of NSIIM and control patients per micrograph. Each micrography was calibrated and measured mean density (divide integrated optical density [IOD] by the sum of area).

2.5 | Statistical analysis

All experiments were performed at least three times. Statistical analysis was performed with GraphPad Prism 9 (GraphPad Software). Values are expressed as means ± standard error of the mean (SEM). A comparison of variables' means between groups was performed using the Mann–Whitney U test. $p < 0.05$ was considered as statistically significant difference.

3 | RESULTS

3.1 | Clinical characteristics of patients with IIM

The basic clinical and pathological data of IIM patients are summarized in Table 1. Among the 26 patients with IIM, 14 (No. 1–14) had dermatomyositis (DM), 8 (No. 15–22) had immune-mediated necrotizing myopathy (IMNM), and 4 (No. 23–26) had non-specific IIM (NSIIM). Muscle weakness was clinically detected in all patients. The duration of clinical symptoms prior to biopsy ranged from 1 to 36 months. A characteristic skin rash was seen in 13 DM patients.

Perifascicular atrophy and inflammatory infiltrate were identified in 6 and 14 DM patients. All IMNM patients showed prominent myofiber necrosis. The inflammatory infiltrates and necrotic fibers were presented in 3 (No. 24-26) and 1 (No. 25) NSIIM patients.

3.2 | Activation of the cGAS-STING pathway was found in IIM muscle

First, we investigated the expression of the cGAS-STING pathway-associated protein. Immunohistochemical analysis showed that the expression of the cGAS and the STING were increased in patients with IIM compared to healthy patients (Figure 1). Then, the expression was further evaluated using Western blot and qRT-PCR. Western blot suggested that cGAS and STING proteins were

upregulated in IIM compared to healthy patients. Moreover, TBK1, which is recruited by STING, and P-STING, activated by TBK1, were upregulated in IIM. In addition, P-IRF3 and P-TBK1, activated by the cGAS-STING pathway and associated with the production of IFNs, were upregulated in IIM. Also, p-NF-kB, activated by cGAS-STING and associated with the production of TNF-a, was over-expressed in IIM (Figure 2 shows data in IIM patients vs. control samples).

QRT-PCR further confirmed the activation of the cGAS-STING pathway, evidenced by the upregulation of cGAS, STING, and TNF-a mRNA expression in the IIM group versus control group (Figure 3).

Moreover, comparing to the Dys patients, PCR analysis shows that cGAS, STING, and TNF-a were upregulated both in IMNM and DM, and the protein of the TBK1, P-IRF3, and P-TBK1 which participate in the upregulation of IFNs was overexpressed by Western blot.

3.3 | The activation of the cGAS-STING pathway associated with atrophic/necrotic fibers

Next, we further examined the role of the cGAS-STING pathway in IIM. IFN-I, which can be upregulated by the cGAS-STING pathway, can induce myotube atrophy in muscle cells in vitro and has been associated with perifascicular atrophy.[13] Therefore, we next examined whether the protein associated with the cGAS-STING pathway was distributed in the atrophy fibers and their surrounding areas.

Immunohistochemistry indicated that cGAS and STING were expressed in some atrophic muscle fibers and inflammatory infiltrates of PFA and vascular structures in DM muscle specimens (Figure 4). Moreover, some cGAS and STING-positive muscle fibers were also positive for Mx1 in atrophic fibers in the DM patients. In addition, we found that cGAS and STING were also expressed in necrotic fibers and myophagocytosis (the phagocytosis of myofibers by macrophages) as well as in regenerating fibers of IMNM patients (Figure 5). In NSIIM, the protein of cGAS and STING was deposited only in inflammatory infiltrates and vascular structures (Figure 1A). In contrast, muscle biopsies from non-disease controls showed negative cGAS and STING staining in fibers (Figure 1A).

We also found some lower cGAS and STING expression in atrophic and necrosis fibers in skeletal muscle biopsy of one patient diagnosed with BMD through immunohistochemistry, while no cGAS and STING were seen in the other three patients with muscular dystrophy.

4 | DISCUSSION

Our data suggested that the cGAS-STING pathway was activated in patients with IIM. We found that cGAS and STING were located in a vascular structure, inflammatory infiltrates, and astrophic and necrotic muscle fibers. In addition, our results suggested that the activation of cGAS, STING, and MxI was associated with atrophic muscles in DM. Therefore, the activation of the cGAS-STING pathway
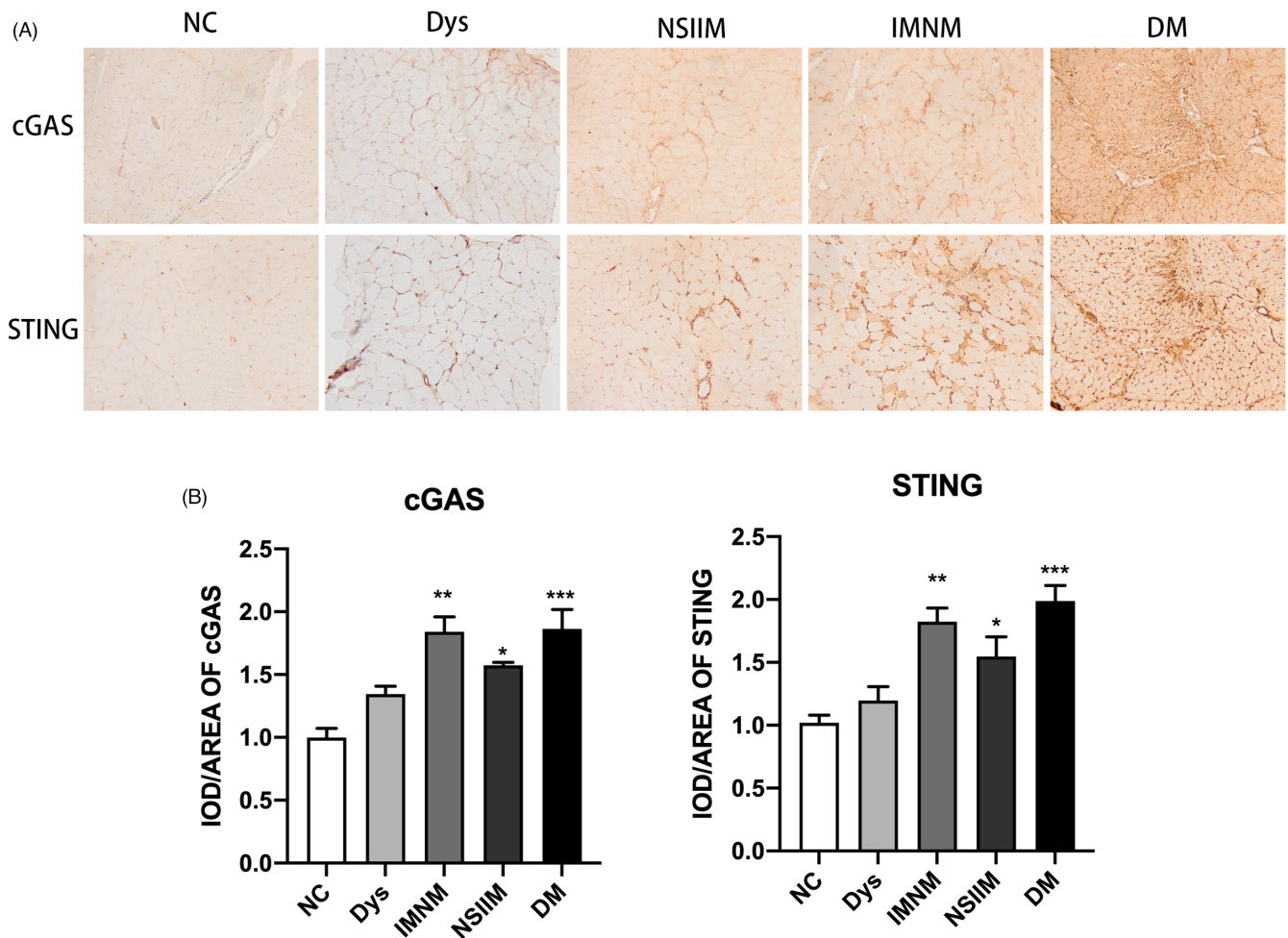
(A)

(B)

FIGURE 1 Immunohistochemical analysis of cGAS and STING in muscle biopsies of IIM patients. (A) cGAS and STING were upregulated in IIM compared to healthy patients. x200. (B) Compared with the control samples (NC), IIM muscle specimens had significantly increased expression level of cGAS and STING. *Significantly different from NC. *$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, ****$p \leq 0.00001$. DM, dermatomyositis; Dys, dystrophinopathy; IMNM, immune-mediated necrotizing myopathy; IOD, integrated optical density; NC, normal control; NSIIM, non-specific IIM.

was associated with atrophic/necrotic muscle in DM and IMNM patients.

DM is a multisystem, immune-mediated disorder that prominently involves specific skin rashes. The pathognomonic histologic feature of perifascicular atrophy (PFA) in skeletal muscle biopsies is considered a hallmark of DM. Recently, more evidence has revealed that IFN-I pathway activation has a critical role in DM pathogenesis. Activation of IFN-I pathway reproduces the upregulation of proteins such as ISG15,[30] Mx1[12] in DM muscle biopsies. In vivo data further suggested that type I IFN signature is correlated with muscle atrophy[13] and mitochondrial dysfunction.[31] Moreover, the expression level of IFN I-inducible genes correlates with indicators of DM disease activity.[7,8] However, the source of the IFN-I signature has not yet been resolved.

Overexpression of innate immune receptors in DM myofibers, such as RIG-I[32,33] and TLRs,[34] may participate in IFN-I production. In our study, we found the expression of cGAS and STING proteins were in the perifascicular atrophy area, both perimysiun=m atrophy

and inflammatory infiltrates, which were positive for Mx1, as compared to control patients, which inferred that overexpression of cGAS-STING pathway upregulated the expression IFN-I may be involved in the pathogenesis of atrophic fibers in DM.

On the other hand, IFNs, from the activated cGAS-STING pathway, may also contribute to mitochondrial dysfunction, leading to abnormal distribution of mitochondrial DNA (mtDNA). So, the abnormal distribution of double-stranded DNA (dsDNA), including mtDNA, further activates the cGAS-STING pathway, inducing continued production of IFNs and tissue damage to sustain disease duration. Although this process warrants further confirmation in vitro and in vivo, there is no doubt that the cGAS-STING pathway was associated with the mechanism of perifascicular atrophy.

Skeletal muscle biopsies obtained from patients with IMNM show prominent myofiber necrosis and regeneration as well as a scarcity of inflammatory infiltrates.[35,36] Necrotic myofibers exhibit the characteristics of different stages of necrotic and regenerating myofibers. In this study, we observed that both atrophic
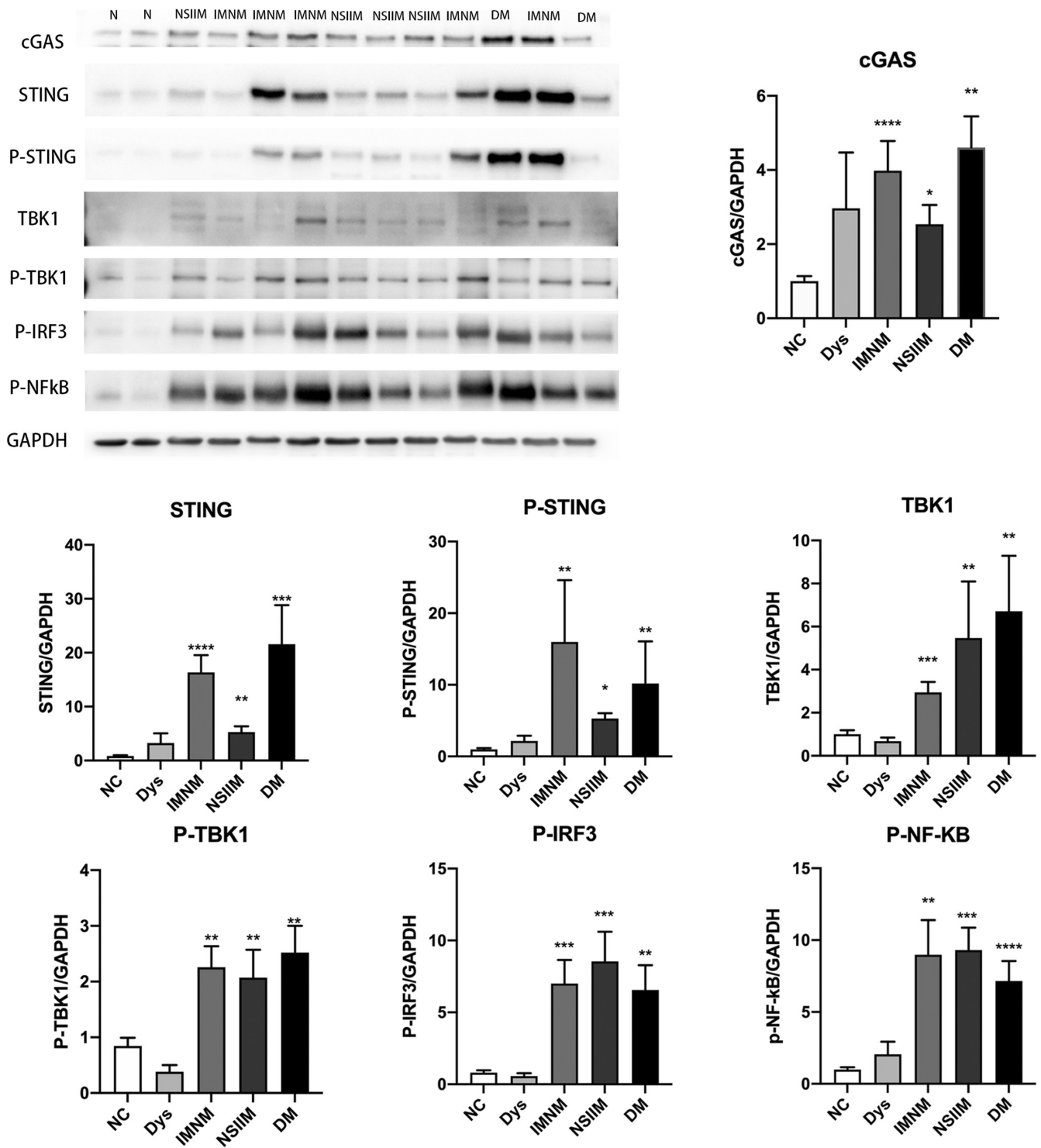
FIGURE 2 cGAS-STING pathway members (cGAS, STING, P-STING, TBK, P-IRF3, P-TBK1, and P-NFkB) in the muscle of IIM examined by Western blot. IIM vs. controls. Data are expressed as means ± SEM. *Significantly different from NC. *$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, ****$p \leq 0.00001$.

and necrotic fibers in IMNM patients were positive of cGAS and STING.

Previous studies suggested a moderated activation of IFN pathway in patients with IMNM.[3] Atrophic fibers in IMNM may be associated with activated IFN pathway, which is consistent with the mechanism of PFA in DM. Considering necrosis, it may be associated with interferon-r and TNF-a. The IFN signature appears to be mainly driven by IFN-II rather than IFN-I, with IFI30 as the most upregulated IFN-inducible gene in IMNM.[3] IFN-r, an IFN-II cytokine produced by TH1 cells, was significantly upregulated in IMNM. TNF-a is a pro-inflammatory cytokine that participates in the development of necrosis.[37] Previous studies showed that TNF-a was significantly
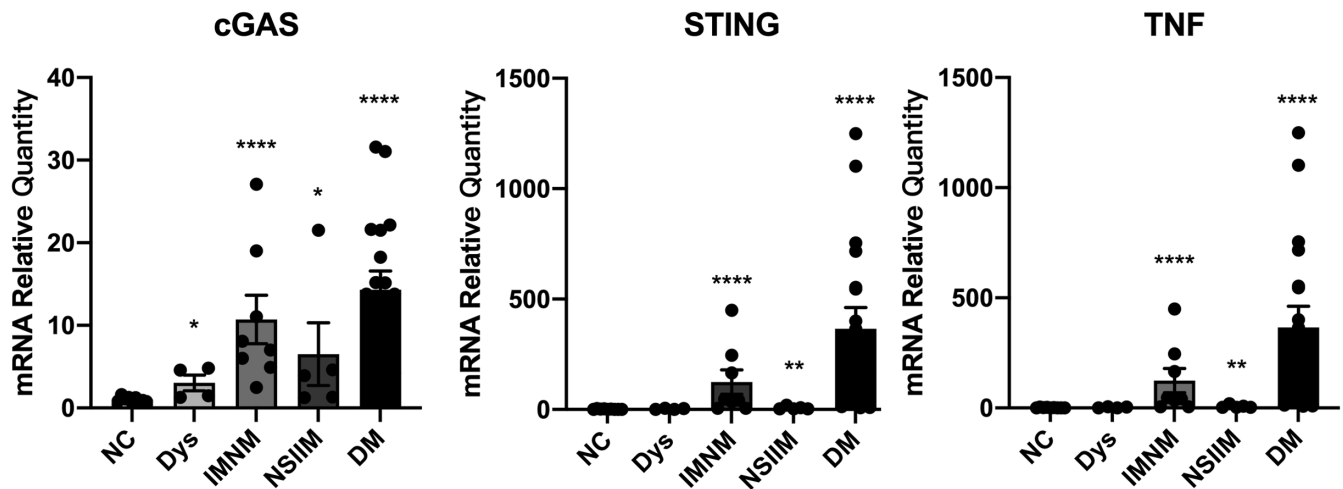
FIGURE 3 qRT-PCR analysis of cGAS-STING pathway-associated molecules in the muscle of IIM. The mRNA expression of cGAS, STING, and TNF-a was all upregulated in IIM. IIM vs. controls. Data are expressed as means ± SEM. *Significantly different from NC. *$p ≤ 0.05$, **$p ≤ 0.001$, ***$p ≤ 0.0001$, ****$p ≤ 0.00001$

FIGURE 4 Expression of cGAS, STING, and MX1 in DM muscle by immunohistochemistry. (A) Representative images of hematoxylin–eosin (HE)-stained sections showing perifascicular atrophy and inflammatory infiltration. (C) cGAS and (D) STING located in some atrophic muscle fibers that (B) MX1 was expressed in the same fibers, and inflammatory infiltrate of PFA and vascular structures in serial sections of a representative dermatomyositis biopsy. x200.
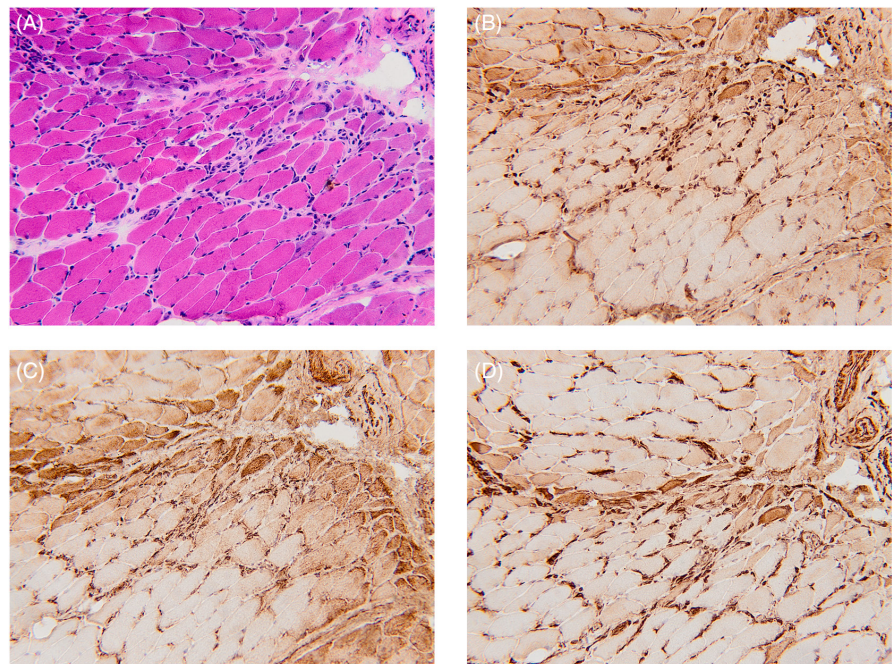
upregulated in IMNM compared with healthy controls.[15,38] Our study also demonstrated that the expression of TNF-a mRNA level was upregulated. IFN-r and TNF-a can be produced by the cGAS-STING pathway. Previous reports have concluded that the activation of the cGAS-STING pathway is related to necrosis.[39,22] In 2018, Brian et al. revealed that TNF-a was more likely required for acute necrosis when the STING pathway in stromal cells, such as fibroblasts and endothelial cells, is activated.[40] Moreover, IFN-r showed a remarkable synergistic effect on TNF-a, such as increasing the number of TNF receptors on the surface of a target cell in tumor treatment.[41] Therefore, it could be inferred that the synergistic effect of IFN-r and TNF-a leads to fiber necrosis in IMNM. On the other hand, numerous studies had confirmed that the cGAS-STING pathway was

associated with cell death, but future studies are required to elucidate these mechanisms and to understand the relationship between fiber necrosis and the cGAS-STING pathway.

The activation of the cGAS-STING pathway was also found in NSIIM patients. The protein of cGAS and STING was located in the inflammatory infiltrates and vascular structure in IIM including NSIIM, IMNM, and DM. Since it was previously proved that activation of the cGAS-STING pathway induced type I interferon response in human monocyte-derived cells,[42] the activation of the cGAS-STING pathway and the production of IFNs may also appear in these inflammatory cells. As cGAS and STING were positive in vascular structure, further studies are needed to explain the relationship between the cGAS-STING pathway and vessels.
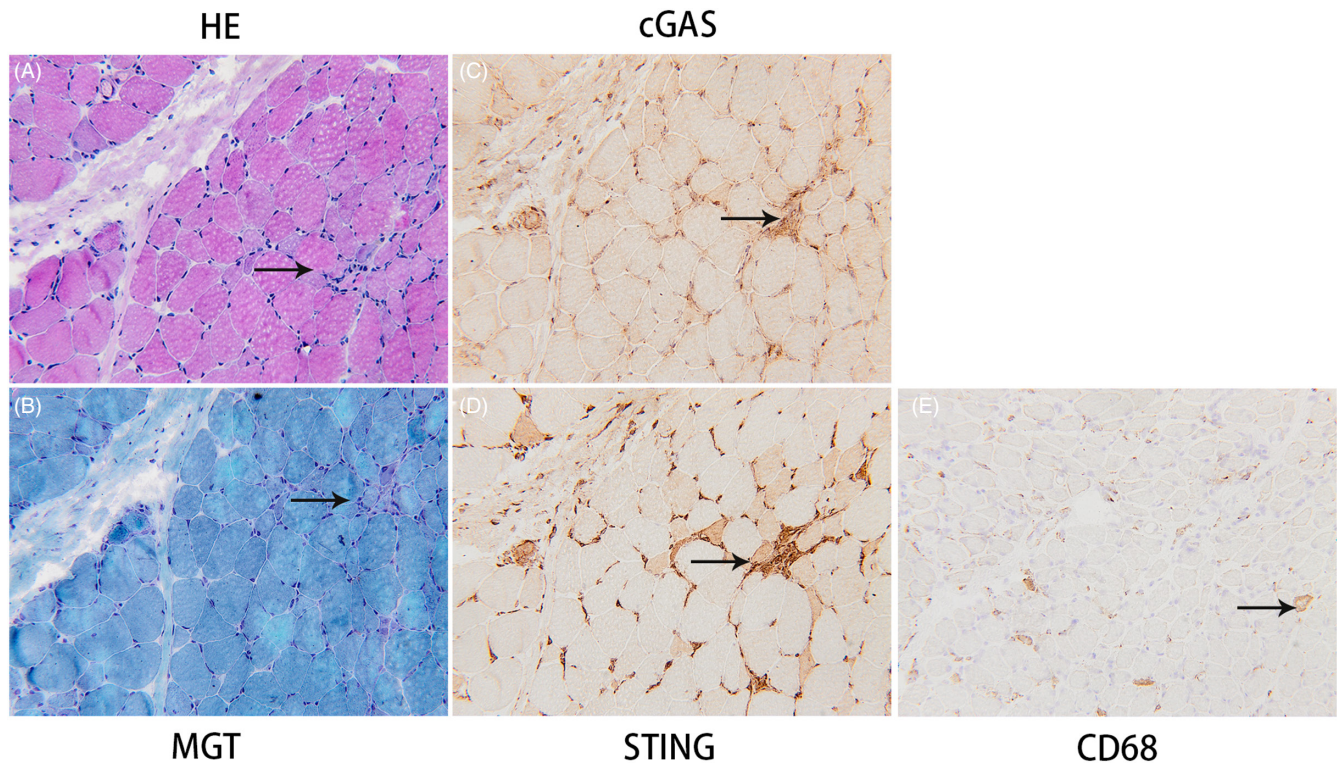
FIGURE 5 Expression of cGAS and STING in IMNM muscle by immunohistochemistry. (A) HE and (B) MGT staining showing necrosis and regeneration of muscle fibers. (C) cGAS and (D) STING were expressed in atrophic fibers, necrotic fibers, and myophagocytosis according to (E) CD68 staining. x400.

This study has a few limitations. First, protein levels of IFNs and TNF-a were not directly deleted. Second, in this study, no in vivo and in vitro were performed to certify the cGAS-STING pathway involved in the pathological process of atrophic and necrotic myofibers. Last, the number of included patients is very limited.

In conclusion, the activation of cGAS-STING pathway was associated with the atrophic/necrotic fibers. Further studies are needed to reveal the role of cGAS-STING pathway in DM and IMNM. This will provides a new idea for understanding pathogenesis and treatment.

CONFLICT OF INTEREST
All authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Meichen Zhou https://orcid.org/0000-0002-2394-6419

REFERENCES
1. Selva-O'Callaghan A, Pinal-Fernandez I, Trallero-Araguás E, Milisenda JC, Grau-Junyent JM, Mammen AL. Classification and management of adult inflammatory myopathies. *Lancet Neurol.* 2018;17(9):816-828. doi:10.1016/s1474-4422(18)30254-0
2. De Paepe B. Interferons as components of the complex web of reactions sustaining inflammation in idiopathic inflammatory myopathies. *Cytokine.* 2015;74(1):81-87. doi:10.1016/j.cyto.2014.10.012
3. Pinal-Fernandez I, Casal-Dominguez M, Derfoul A, et al. Identification of distinctive interferon gene signatures in different types of myositis. *Neurology.* 2019;93(12):e1193-e1204. doi:10.1212/WNL.0000000000008128
4. Greenberg SA, Higgs BW, Morehouse C, et al. Relationship between disease activity and type 1 interferon- and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. *Genes Immun.* 2012;13(3):207-213. doi:10.1038/gene.2011.61
5. Ekholm L, Vosslamber S, Tjarnlund A, et al. Autoantibody specificities and type I interferon pathway activation in idiopathic

inflammatory myopathies. *Scand J Immunol.* 2016;84(2):100-109. doi:10.1111/sji.12449

6. Greenberg SA, Pinkus JL, Pinkus GS, et al. Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. *Ann Neurol.* 2005;57(5):664-678. doi:10.1002/ana.20464

7. Walsh RJ, Kong SW, Yao Y, et al. Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. *Arthritis Rheum.* 2007;56(11):3784-3792. doi:10.1002/art.22928

8. Baechler EC, Bauer JW, Slattery CA, et al. An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. *Mol Med.* 2007;13(1–2):59-68. doi:10.2119/2006-00085.Baechler

9. Wong D, Kea B, Pesich R, et al. Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. *PLoS One.* 2012;7(1):e29161. doi:10.1371/journal.pone.0029161

10. Arshanapalli A, Shah M, Veerula V, Somani AK. The role of type I interferons and other cytokines in dermatomyositis. *Cytokine.* 2015;73(2):319-325. doi:10.1016/j.cyto.2014.11.026

11. Bolko L, Jiang W, Tawara N, et al. The role of interferons type I, II and III in myositis: a review. *Brain Pathol.* 2021;31(3):e12955. doi:10.1111/bpa.12955

12. Uruha A, Allenbach Y, Charuel JL, et al. Diagnostic potential of sarcoplasmic myxovirus resistance protein a expression in subsets of dermatomyositis. *Neuropathol Appl Neurobiol.* 2019;45(5):513-522. doi:10.1111/nan.12519

13. Ladislau L, Suarez-Calvet X, Toquet S, et al. JAK inhibitor improves type I interferon induced damage: proof of concept in dermatomyositis. *Brain.* 2018;141(6):1609-1621. doi:10.1093/brain/awy105

14. Kahn JS, Deverapalli SC, Rosmarin DM, Wengert O. JAK-STAT signaling pathway inhibition: a role for treatment of discoid lupus erythematosus and dermatomyositis. *Int J Dermatol.* 2018;57(8):1007-1014. doi:10.1111/ijd.14064

15. Preuße C, Goebel HH, Held J, Wengert O, et al. Immune-mediated necrotizing myopathy is characterized by a specific Th1-M1 polarized immune profile. *Musculoskeletal Pathol.* 2012;181(6):2161-2171. doi:10.1016/j.ajpath.2012.08.033

16. Zhou L, Zhang YF, Yang FH, Mao HQ, Chen Z, Zhang L. Mitochondrial DNA leakage induces odontoblast inflammation via the cGAS-STING pathway. *Cell Commun Signal.* 2021;19(1):58. doi:10.1186/s12964-021-00738-7

17. Bai J, Cervantes C, Liu J, et al. DsbA-L prevents obesity-induced inflammation and insulin resistance by suppressing the mtDNA release-activated cGAS-cGAMP-STING pathway. *Proc Natl Acad Sci USA.* 2017;114(46):12196-12201. doi:10.1073/pnas.1708744114

18. Qiao JT, Cui C, Qing L, et al. Activation of the STING-IRF3 pathway promotes hepatocyte inflammation, apoptosis and induces metabolic disorders in nonalcoholic fatty liver disease. *Metabolism.* 2018;81:13-24. doi:10.1016/j.metabol.2017.09.010

19. Chung KW, Dhillon P, Huang S, et al. Mitochondrial damage and activation of the STING pathway Lead to renal inflammation and fibrosis. *Cell Metab.* 2019;30(4):784-799.e5. doi:10.1016/j.cmet.2019.08.003

20. Maekawa H, Inoue T, Ouchi H, et al. Mitochondrial damage causes inflammation via cGAS-STING signaling in acute kidney injury. *Cell Rep.* 2019;29(5):1261-1273 e6. doi:10.1016/j.celrep.2019.09.050

21. Quinn GZ, Dhillon P, Susztak K. It takes two to tango: the role of dysregulated metabolism and inflammation in kidney disease development. *Semin Nephrol.* 2020;40(2):199-205. doi:10.1016/j.semnephrol.2020.01.010

22. Zhang R, Kang R, Tang D. The STING1 network regulates autophagy and cell death. *Signal Transduct Target Ther.* 2021;6(1):208. doi:10.1038/s41392-021-00613-4

23. Murthy AMV, Robinson N, Kumar S. Crosstalk between cGAS-STING signaling and cell death. *Cell Death Differ.* 2020;27(11):2989-3003. doi:10.1038/s41418-020-00624-8

24. Liu D, Wu H, Wang C, et al. STING directly activates autophagy to tune the innate immune response. *Cell Death Differ.* 2019;26(9):1735-1749. doi:10.1038/s41418-018-0251-z

25. Ma R, Ortiz Serrano TP, Davis J, Prigge AD, Ridge KM. The cGAS-STING pathway: the role of self-DNA sensing in inflammatory lung disease. *FASEB J.* 2020;34(10):13156-13170. doi:10.1096/fj.202001607R

26. Amadio R, Piperno GM, Benvenuti F. Self-DNA sensing by cGAS-STING and TLR9 in autoimmunity: is the cytoskeleton in control? *Front Immunol.* 2021;12:657344. doi:10.3389/fimmu.2021.657344

27. Li YX, Cui SF, Meng W, Hu HY, Wang C. Mitochondrial DNA and cGAS-STING innate immune signaling pathway: latest research Progress. *Sichuan Da Xue Xue Bao Yi Xue Ban.* 2021;52(3):387-395. doi:10.12182/20210560501

28. Yu CH, Davidson S, Harapas CR, et al. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. *Cell.* 2020;183(3):636-649.e18. doi:10.1016/j.cell.2020.09.020

29. Hoogendijk JE, Amato AA, Lecky BR, et al. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10–12 October 2003, Naarden, The Netherlands. *Neuromuscul Disord.* 2004;14(5):337-345. doi:10.1016/j.nmd.2004.02.006

30. Salajegheh M, Kong SW, Pinkus JL, et al. Interferon-stimulated gene 15 (ISG15) conjugates proteins in dermatomyositis muscle with perifascicular atrophy. *Ann Neurol.* 2010;67(1):53-63. doi:10.1002/ana.21805

31. Meyer A, Laverny G, Allenbach Y, et al. IFN-beta-induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in dermatomyositis. *Acta Neuropathol.* 2017;134(4):655-666. doi:10.1007/s00401-017-1731-9

32. Suarez-Calvet X, Gallardo E, Nogales-Gadea G, et al. Altered RIG-I/DDX58-mediated innate immunity in dermatomyositis. *J Pathol.* 2014;233(3):258-268. doi:10.1002/path.4346

33. De Luna N, Suarez-Calvet X, Lleixa C, et al. Hypoxia triggers IFN-I production in muscle: implications in dermatomyositis. *Sci Rep.* 2017;7(1):8595. doi:10.1038/s41598-017-09309-8

34. Li L, Dai T, Lv J, et al. Role of toll-like receptors and retinoic acid inducible gene I in endogenous production of type I interferon in dermatomyositis. *J Neuroimmunol.* 2015;285:161-168. doi:10.1016/j.jneuroim.2015.06.009

35. Allenbach Y, Mammen AL, Benveniste O, Stenzel W, Immune-Mediated Necrotizing Myopathies Working G. 224th ENMC international workshop:: Clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14–16 October 2016. *Neuromuscul Disord.* 2018;28(1):87-99. doi:10.1016/j.nmd.2017.09.016

36. Ma X, Xu L, Ji S, Li Y, Bu B. The clinicopathological distinction between seropositive and seronegative immune-mediated necrotizing myopathy in China. *Front Neurol.* 2021;12:670784. doi:10.3389/fneur.2021.670784

37. Dunbar JP, Sulpice R, Dugon MM. The kiss of (cell) death: can venom-induced immune response contribute to dermal necrosis following arthropod envenomations? *Clin Toxicol (Phila).* 2019;57(8):677-685. doi:10.1080/15563650.2019.1578367

38. Chen X, Li W, Ren J, et al. Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. *Cell Res.* 2014;24(1):105-121. doi:10.1038/cr.2013.171

39. Liu S, Guan W. STING signaling promotes apoptosis, necrosis, and cell death: an overview and update. *Mediators Inflamm.* 2018;2018:1202797-1202794. doi:10.1155/2018/1202797

40. Francica BJ, Ghasemzadeh A, Desbien AL, et al. TNFα and radio-resistant stromal cells are essential for therapeutic efficacy of

cyclic dinucleotide STING agonists in nonimmunogenic tumors. *Cancer Immunol Res.* 2018;6(4):422-433. doi:10.1158/2326-6066. Cir-17-0263

41. Guan YQ, Zheng Z, Li Z, Liu JM. Cell death in HeLa mediated by thermoplastic polyurethane with co-immobilized IFN-gamma plus TNF-alpha. *Acta Biomater.* 2012;8(3):1348-1356. doi:10.1016/j. actbio.2011.11.023

42. Paijo J, Doring M, Spanier J, et al. cGAS senses human cytomegalovirus and induces type I interferon responses in human monocyte-derived cells. *PLoS Pathog.* 2016;12(4):e1005546. doi:10.1371/ journal.ppat.1005546