J Neuropathol Exp Neurol
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Vol. 68, No. 3
March 2009
pp. 262–273

ORIGINAL ARTICLE

TAR DNA-Binding Protein 43 Accumulation in Protein Aggregate Myopathies

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Abstract

Protein aggregate myopathies, including myofibrillar myopathies and sporadic inclusion body myositis (sIBM), are characterized by abnormal protein aggregates composed of various muscular and ectopic proteins. Previous studies have shown the crucial role of dysregulated transcription factors such as neuron-restrictive silencer factor in the expression of aberrant proteins in myotilinopathies. Here, we assessed possible aberrant expression of TAR DNA-binding protein 43 (TDP-43), another factor involved in transcription regulation. TDP-43-immunoreactive intracytoplasmic inclusions were seen in all cases examined of myotilinopathy, desminopathy, and sIBM, and in 1 case of inclusion body myositis with Paget disease of bone and frontotemporal degeneration (IBMPFD). TAR DNA-binding protein 43 colocalized with myotilin and valosin in myotilinopathies and IBMPFD, respectively, but only occasionally colocalized with ubiquitin in myotilinopathies, desminopathies, sIBM, and IBMPFD; this indicates that accumulated TDP-43 is largely not ubiquitinated. Moreover, phosphorylated TDP-43 at Ser403/404 and Ser409/410 accumulated in the cytoplasm of vulnerable fibers but did not always colocalize with nonphosphorylated TDP-43. Cytoplasmic deposition was accompanied by decreased TDP-43 localization in the nuclei of affected fibers. These findings indicate that TDP-43 not only is another protein accumulated in myofibrillar myopathies, sIBM, and IBMPFD but also likely has a role through altered microRNA processing in the abnormal protein production, modification, and accumulation in protein aggregate myopathies.

Key Words: Desminopathy, Inclusion body myositis with Paget disease of bone and frontotemporal dementia, Myotilinopathy, Sporadic inclusion body myositis, TDP-43, Valosin.

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This study was supported by an FIS grant PI080574.

INTRODUCTION

Intracellular accumulation of proteins is characteristic of several diverse human disorders. Protein aggregation has been recognized for a long time as a major morphologic hallmark of many neurodegenerative disorders (1–4) and is now also considered an important pathogenetic factor in a growing group of muscle disorders collectively termed "protein aggregate myopathies" (PAM) (5, 6). Myofibrillar myopathies (MFMs), the largest group of PAMs, are a group of heterogeneous muscle disorders having as a common feature the presence of focal dissolution of the myofibrils and ectopic expression of multiple proteins (7–10). Among MFMs, myotilinopathies and desminopathies are 2 genetically distinct subgroups caused by mutations in *MYOT* and *DES* genes, respectively (12–14). Protein aggregates in MFMs are composed of a wide variety of proteins (7–10). Some of them, such as desmin, myotilin, and dystrophin, are normal constituents of the myofibrils, but many proteins not specific to muscle are aberrantly expressed in muscle fibers as well. These include phosphorylated tau, ubiquitin, β-amyloid, and diverse neuronal proteins such as synaptophysin, SNAP25, α-internexin, and ubiquitin carboxy-terminal hydrolase L1 (7–10, 15). Sporadic inclusion body myositis (sIBM) represents the nongenetic counterpart of protein aggregate myopathies, and it is characterized by the presence of ubiquitinated inclusion bodies containing β-amyloid, α-synuclein, apolipoprotein E, and phosphorylated tau among others (16, 17).

Recent studies have shown the crucial role of certain transcription factors in the pathogenesis of MFMs. Target genes of neuron-restrictive silencer factor are abnormally upregulated in human myotilinopathy due to the reduced levels of neuron-restrictive silencer factor, a transcription factor expressed in nonneuronal tissues that represses the expression of several neuronal genes. As a consequence, synaptophysin, SNAP25, α-internexin, and ubiquitin carboxy-terminal hydrolase L1 are aberrantly produced and accumulated in protein aggregates in myotilinopathies (15).

Another potentially interesting transcription factor in the pathogenesis of PAMs and of MFMs in particular is TAR DNA-binding protein 43 (TDP-43), a 414-amino acid nuclear protein encoded by the *TARDBP* gene on chromosome 1. The gene was first cloned from a genomic screen for cellular factors that bind to the TAR DNA of human immunodeficiency virus type 1, and it was later identified as part of a complex

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involved in the splicing of the cystic fibrosis transmembrane regulator gene (18–20). TAR DNA-binding protein 43 is highly conserved and widely expressed in several tissues, including brain and muscle. It contains 2 RNA recognition motifs and a glycine-rich C-terminal sequence that is required for exon skipping and splicing inhibitory activity. The C-terminal domain binds to several ribonucleoproteins involved in the biogenesis of mRNA. Although the biologic functions of TDP-43 are not fully known, it has a role in exon skipping, transcription regulation, and other biologic processes through its binding to DNA, RNA, and/or proteins (18–21).

Recent studies have identified TDP-43 as the major protein forming insoluble aggregates in neurons and glial cells in most patients with frontotemporal lobar degeneration with ubiquitin inclusions (FTLD-U) with and without motor neuron disease, as well as in sporadic and familial amyotrophic lateral sclerosis (ALS) not caused by mutations in *SOD1* gene (22–25). Among the heterogeneous group of FTLD-U, up to 40% of cases show a familial pattern of inheritance and mutations in progranulin, and valosin-containing protein (VCP) have been recently described (26–29).

Mutations in the *VCP* gene, on chromosome 9p13.3-p12, cause hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD), a rare autosomal dominant disorder with a high variable expressivity of clinical symptoms (28–32). Ubiquitin-positive inclusions in brains in IBMPFD contain TDP-43 (32). TAR DNA-binding protein 43 accumulation has also been described in muscle fibers in IBMPFD (33). Abnormal TDP-43 in muscle fibers in IBMPFD seems not to be related to VCP mutations because TDP-43–immunoreactive cytoplasmic inclusions also occur in sIBM (33).

The aim of the present study was to analyze the expression of TDP-43 in muscle disorders characterized by the presence of protein aggregates in muscle cells. Muscle biopsies from patients who have myotilinopathy, desminopathy, sIBM, and IBMPFD, as well as samples from patients who have denervation atrophy showing target lesions, were examined with single and double labeling immunofluorescence and confocal microscopy and Western blotting to demonstrate possible modifications in the localization, distribution, and expression levels of TDP-43 in muscle cells. TAR DNA-binding protein 43, desmin, and myotilin gene expression was performed to investigate whether increased protein levels result from upregulation of the respective genes.

MATERIALS AND METHODS

Patients and Muscle Biopsies

Muscle biopsies from 9 patients with MFM (5 myotilinopathies and 4 desminopathies), 5 patients with sIBM, and 1 patient with IBMPFD were studied. Muscle samples from 5 cases of denervation atrophy that showed target lesions and 5 age-matched healthy controls were processed in parallel. Table 1 summarizes the clinical characteristics of the patients. The patients with myotilinopathy were 3 men and 2 women aged 49 to 81 years (mean, 65.8 years); they had the following *MYOT* mutations: Ser55Phe, Ser60Cys, Ser60Phe, and Lys36Glu. There were 2 female and 2 male desminopathy patients aged 22 to 55 years (mean, 36.5 years) with the following *DES* mutations: Pro419Ser, Leu392Pro, Ile367Phe, and Arg406Trp. Detailed clinical, pathologic, and genetic characterization of most of these patients has been described elsewhere (12, 34). There were 3 male and 2 female patients with sIBM aged 58 to 66 years (mean, 61.5 years). The diagnosis was established on the basis of well-established clinical and histopathologic criteria (17). The patient with IBMPFD was a 57-year-old man with proximal and distal muscle weakness in 4 limbs and scapular winging from the age of 49 years. He had no clinical, laboratory, or radiologic evidence of Paget disease of bone and had no cognitive symptoms. Sequencing analysis of *VCP* gene identified a R159H mutation. The patient's father had progressive muscle weakness starting at approximately 60 years and had died 5 years later of a heart attack. No other members of the family were known to be affected by muscle weakness, dementia, or Paget disease of bone.

In all cases, muscle biopsies were obtained for diagnostic purposes after informed consent and according to the guidelines of the Ethics Committee of the Hospital Universitari de Bellvitge. All samples had previously been examined by routine histochemical and electron microscopy techniques and by immunocytochemical analysis. Fresh frozen and mounted frozen samples were kept at -80°C until used.

TABLE 1. Summary of Cases

Patient	Age (years)/Sex	Diagnosis
1	52/M	Myotilinopathy (Ser60Cys)
2	49/M	Myotilinopathy (ser55Phe)
3	78/M	Myotilinopathy (Ser60Phe)
4	81/F	Myotilinopathy (Lys36Glu)
5	69/F	Myotilinopathy (Ser60Cys)
6	41/F	Desminopathy (Pro419Ser)
7	55/F	Desminopathy (Leu 392Pro)
8	28/M	Desminopathy (Ile367Phe)
9	22/M	Desminopathy (Arg406Trp)
10	61/M	IBM
11	61/F	IBM
12	62/M	IBM
13	66/M	IBM
14	58/F	IBM
15	57/M	IBMPFD (Arg159His)
16	76/M	Denervation atrophy
17	32/F	Denervation atrophy
18	56/M	Denervation atrophy
19	65/M	Denervation atrophy
20	67/F	Denervation atrophy
21	57/M	Control
22	63/M	Control
23	43/F	Control
24	30/M	Control
25	70/M	Control

F, female; IBM, inclusion body myositis; IBMPFD, inclusion body myositis with Paget disease of bone and frontotemporal degeneration; M, male.

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Unfixed cryostat sections 6 μm thick were stained with hematoxylin and eosin and modified trichrome stain and processed for immunohistochemistry with the streptavidin-biotin Super Sensitive TM IHC detection system (BioGenex, San Ramon, CA), as previously described (35). Antibodies to myotilin (Novocastra, Servicios Hospitalarios, Barcelona, Spain), desmin (Dako, Barcelona, Spain), ubiquitin (Dako), and valosin (Affinity Bioreagents, Bionova Cientifica, Madrid, Spain) were used at dilutions of 1:100, 1:20, 1:100, and 1:1000, respectively (Table 2).

TDP-43 Immunofluorescence

TAR DNA-binding protein 43 was examined using 2 different antibodies: a mouse monoclonal antibody (Abnova, Tebu-Bio, Barcelona, Spain; H00023435-M01) raised against a full-length recombinant human TARDBP, used at a dilution of 1:1000, and a rabbit polyclonal antibody (Abcam, Cambridge, UK; ab54502) raised against a synthetic peptide corresponding to C terminal (aa 350-414) of human TARDBP, used at a dilution of 1:2000. Phospho–TDP-43 was studied using 2 different antibodies: a mouse monoclonal antibody directed to CMDSKS(p)S(p)GWGM,S(p), Ser409/410, used at a dilution of 1:5000, and a rabbit polyclonal antibody raised against NGGFGS(p)S(p)MDSKC,S(p), Se403/404, used at a dilution of 1:2500 (both from Cosmo Bio, Ltd., Koto-ku, Japan) (Table 2). The secondary antibodies Alexa 488 and Alexa 455 (Molecular Probes, Invitrogen, Madrid, Spain) were used at a dilution of 1:400. Sections were mounted with Fluorescent Mounting Medium (DakoCytomation, Barcelona, Spain), sealed, and dried overnight at 4°C.

Double Labeling Immunofluorescence and Confocal Microscopy

Cryostat sections 8 μm thick were blocked for 30 minutes at room temperature with 10% fetal bovine serum diluted in 1 × PBS to avoid nonspecific binding. Sections were incubated overnight at 4°C with different combinations of 2 primary antibodies as follows: 1) mouse monoclonal anti-myotilin antibody or mouse monoclonal anti-desmin antibody was used as the first primary antibody and rabbit polyclonal anti–TDP-43 antibody as a second primary antibody; 2) rabbit polyclonal anti-ubiquitin antibody and mouse monoclonal anti–TDP-43 antibody. In samples of sIBM and IBMPFD, the following combinations were made: 1) mouse monoclonal anti–TDP-43 antibody and goat polyclonal anti-valosin antibody; 2) mouse monoclonal anti–TDP-43 antibody and rabbit polyclonal anti-ubiquitin antibody; 3) rabbit polyclonal anti-ubiquitin antibody and mouse monoclonal anti–TDP-43 antibody. After washing with PBS, the sections were incubated in a cocktail of secondary antibodies in the same vehicle solution for 3 hours at room temperature. The secondary antibodies were Alexa 488 and Alexa 546 anti-mouse or anti-rabbit (Molecular Probes) at a dilution of 1:400. Double labeling immunofluorescence was also performed using anti–TDP-43 (Abnova and Abcam) and anti–phospho–TDP-43 (Cosmo Bio) antibodies using a parallel combination of rabbit polyclonal and mouse monoclonal antibodies and vice versa. Subsequently, the nuclei were stained using To-pro-3-iodide (Molecular Probes) at a dilution of 1:1000 for 20 minutes at room temperature. Sections were mounted with Fluorescent Mounting Medium (DakoCytomation), sealed, and dried overnight at 4°C. Sections were examined with a Leica TCS-SL confocal microscope. To rule out nonspecific reactions, some sections were incubated only with the secondary antibodies.

Gel Electrophoresis and Western Blotting

Samples from MFM, sIBM, IBMPFD, and control patients were processed for Western blot analysis using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Briefly, extracted muscle proteins were transferred to nitrocellulose membrane in a Semi-Dry Transfer System (Bio-Rad, Madrid, Spain). The corresponding membranes were blocked and incubated with the mouse monoclonal anti–TDP-43 antibody (Abnova) at a dilution of 1:1000. Subsequently, the membranes were washed and then incubated with the corresponding secondary antibody labeled with horseradish peroxidase (Dako). The protein bands were detected by enhanced chemiluminescence method (Amersham Biosciences, Little Chalfont, UK). The myosin band of 205 kDa stained with Coomassie Brilliant Blue R (Sigma, Madrid, Spain) in the posttransfer gel was used as control of protein loading. Densitometric quantification of Western blot bands was performed with Total Lab v2.01 software, and the data obtained were analyzed using Statgraphics Plus v5.1 software. Statistical analysis was performed with the Student *t*-test.

mRNA Isolation and cDNA Synthesis

Total RNA was purified from frozen muscle biopsies using the RNeasy Fibrous Tissue Mini kit (Qiagen, Las

TABLE 2. Antibodies Used in the Present Study

Antigen	Antibody	Species	Source	Dilution (IH;IF)	Dilution (WB)
Myotilin	Monoclonal	Mouse	Novocastra	1:100	—
Desmin	Monoclonal	Mouse	Dako	1:15	—
TDP-43 C terminal	Polyclonal	Rabbit	Abcam (ab54502)	1/2000	—
TDP-43	Monoclonal	Mouse	Abnova, (H00023435-M01)	1/1000	1/1000
Phospho–TDP-43 (ps409/410)	Monoclonal	Mouse	Cosmo Bio	1/5000	—
Phospho–TDP-43 (ps403/404)	Polyclonal	Rabbit	Cosmo Bio	1/2500	—
Valosin	Monoclonal	Mouse	Affinity Bioreagents MA3-004	1/1000	—
Ubiquitin	Polyclonal	Rabbit	Dako Z-458	1/100	—

IF, immunofluorescence; IH, immunohistochemistry; TDP-43, TAR DNA-binding protein 43; WB, Western blotting.

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Matas, Madrid, Spain) following the instructions of the supplier. RNA integrity was assessed by using an Agilent Bioanalyzer 2100 (Agilent, Las Rozas, Spain). Then, total RNA of each sample was reverse-transcribed to a single-stranded cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Madrid, Spain). Parallel reactions lacking MultiScribe Reverse Transcriptase were run as negative controls.

TaqMan MGB Probes and Endogenous Controls

TaqMan Gene Expression Assays (Applied Biosystems) using specific primers and probes designed to detect TDP-43 (Hs00606522_m1) were performed. TAR DNA-binding protein 43 probe was located between the exon 3 and 4 boundary of the NM_007375.3 transcript and produced an amplicon of 130 bp. Two TaqMan endogenous controls were used to normalize TDP-43 expression levels: human β-glucuronidase (GUS; 4333767) and human β-2-microglobulin (B2M; 4333766).

TaqMan Real-Time Polymerase Chain Reaction

TaqMan polymerase chain reaction (PCR) assays for TDP-43 (and desmin and myotilin in MFMs) were performed in duplicate on cDNA samples in a MicroAmp Optical 384-Well Reaction Plate sealed with MicroAmp Optical Adhesive Film (Applied Biosystems). Each 20-μl PCR reaction was prepared with 9 μl of cDNA (diluted 1/10 in MFMs and 1/5 in IBMs, which corresponded to approximately 20 ng of input RNA in both cases) mixed with 1 μl of 20× TaqMan Gene Expression Assay Mix and 10 μl of 2× TaqMan Universal PCR Master

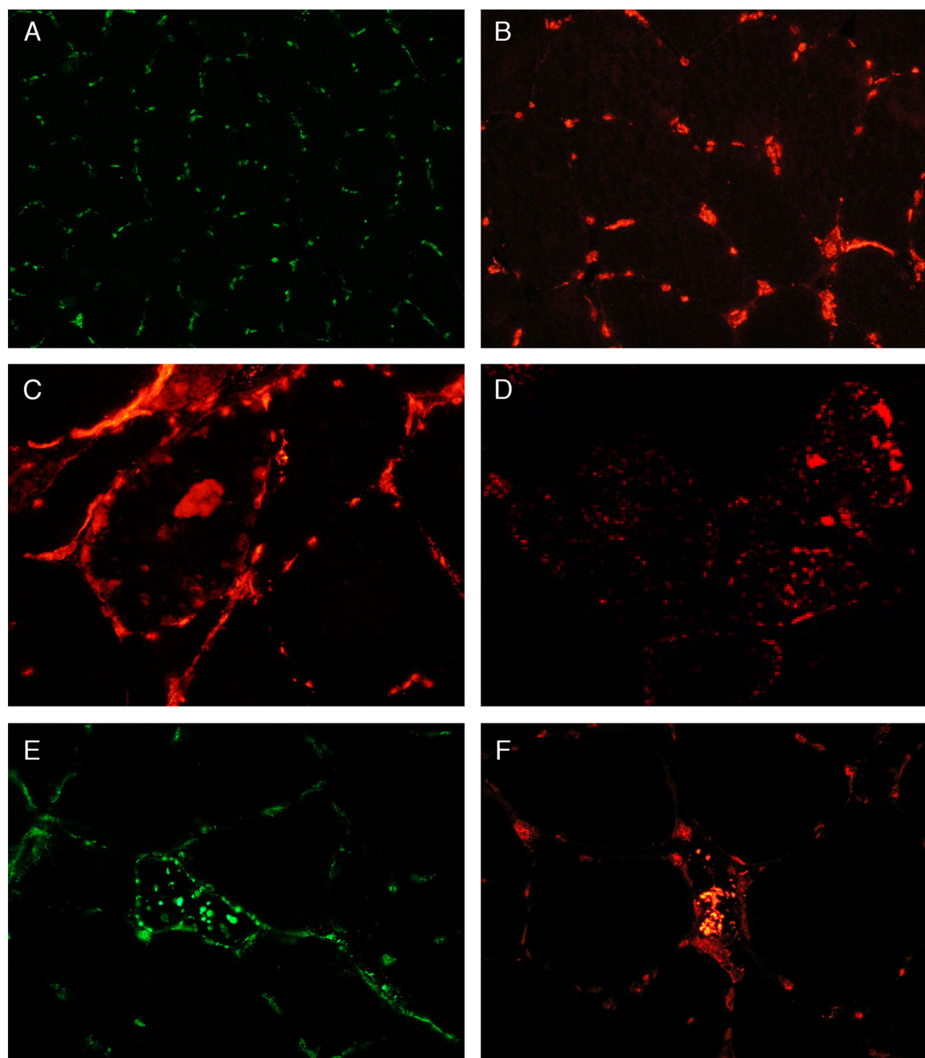

FIGURE 1. TAR DNA-binding protein 43 (TDP-43) immunofluorescence using the rabbit polyclonal antibody against the C-terminus of TDP-43 **(A, C, E)** and the mouse monoclonal antibody recognizing full-length recombinant human TDP-43 **(B, D, F)** in normal muscle **(A, B)**, myotilinopathy **(C)**, desminopathy **(D)**, sporadic inclusion body myositis (sIBM) **(E)**, and inclusion body myositis with Paget disease of bone and frontotemporal degeneration (IBMPFD) **(F)**. TAR DNA-binding protein 43 in normal muscle is restricted to the nuclei, whereas intracytoplasmic accumulation of TDP-43 is seen in myotilinopathy, sIBM, and IBMPFD, and in desminopathy to a lesser degree. Original magnification: **(A)** 100×; **(B, D–F)** 200×; **(C)** 400×.

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Mix (Applied Biosystems), as indicated by the manufacturer. Parallel reactions of all samples were performed in duplicate using GUS and 2BM endogenous control assays for data normalization. Standard curves for each probe used in the study were obtained with serial dilutions of a muscle control sample. The thermal cycler parameters were set up for 2 minutes at 50°C (UNG activation), then 10 minutes at 95°C (enzyme activation), followed by 40 cycles at 95°C for 15 seconds (denaturation) and 1 minute at 60°C (annealing/extension). The fluorescent PCR product was measured with an ABI PRISM 7900HT Fast Sequence Detection System (Applied Biosystems), and the emerging data were captured with the Sequence Detector Software (SDS, 1.9; Applied Biosystems).

Data Processing and Statistical Analysis

ΔCt values for each sample were promediated, and their equivalent amount of RNA were interpolated from the standards curves. These values were normalized, and acquired data were analyzed using Statgraphics Plus v5.1 software. Differences among control and pathologic samples were analyzed with ANOVA, followed by LSD post-hoc test.

RESULTS

TDP-43 Immunostaining in Normal and Diseased Muscle

In control muscles, TDP-43 immunofluorescence was restricted to the nuclei of muscle cells; cytoplasmic immunostaining was absent. Similar results were observed using the 2 different anti–TDP-43 antibodies (Fig. 1A, B).

In contrast, marked TDP-43 accumulation in the form of single, multiple, or diffuse deposits was seen in the cytoplasm of several muscle fibers in myotilinopathy (Fig. 1C). Muscle fibers from desminopathy cases also showed TDP-43–positive inclusions in the cytoplasm. TAR DNA-binding protein 43 accumulation in desminopathies was, however, usually less prominent than in myotilinopathies (Fig. 1D). TAR DNA-binding protein 43 immunostaining was also observed in the cytoplasm of several muscle fibers in all sIBM cases (Fig. 1E)

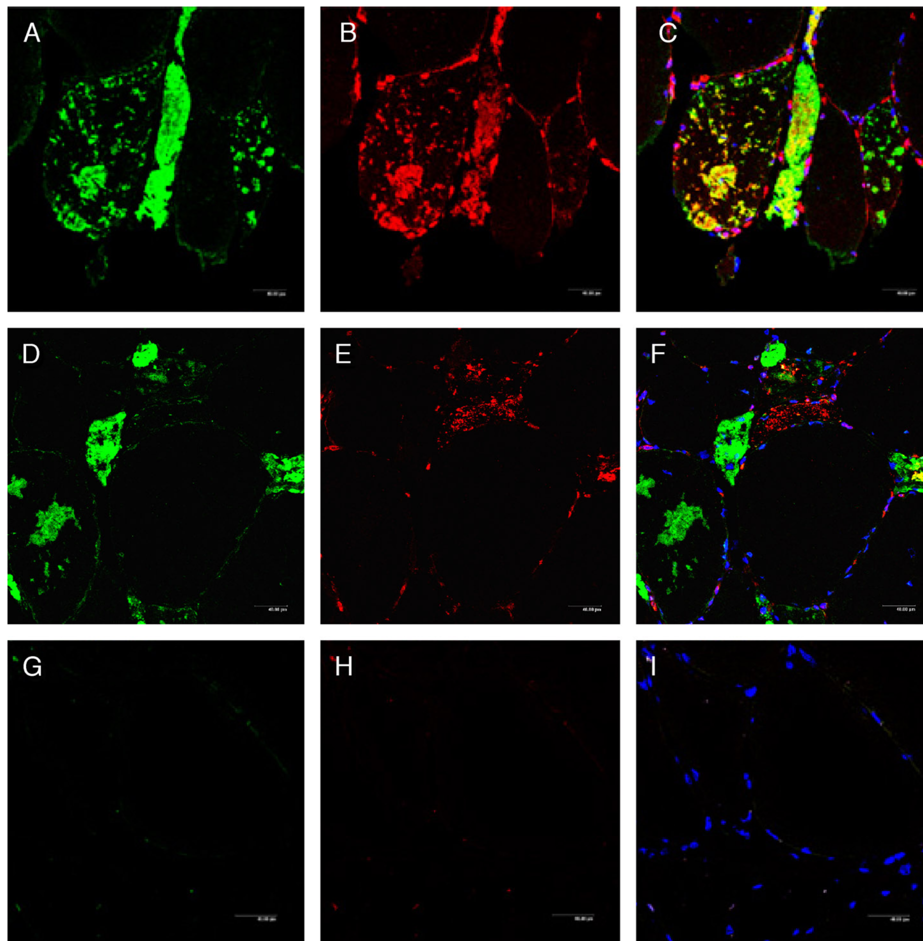

FIGURE 2. Double labeling immunofluorescence and confocal microscopy to myotilin **(**green, **A)** and TAR DNA-binding protein 43 **(**TDP-43; red, **B)**; and ubiquitin **(**green, **D)** and TDP-43 **(**red, **E)** in myotilinopathy. Partial colocalization of myotilin and TDP-43 **(**merge, yellow, **C)** is seen in some inclusions. Ubiquitin and TDP-43 colocalization is much less common **(**merge, yellow, **F)**. One section of the same case stained only with the secondary antibodies is used as a negative control **(G–I)**. Nuclei are visualized with To-pro-3-iodide (blue). Note decreased or absent TDP-43 immunoreactivity in the nuclei of fibers containing abnormal TDP-43 aggregates.

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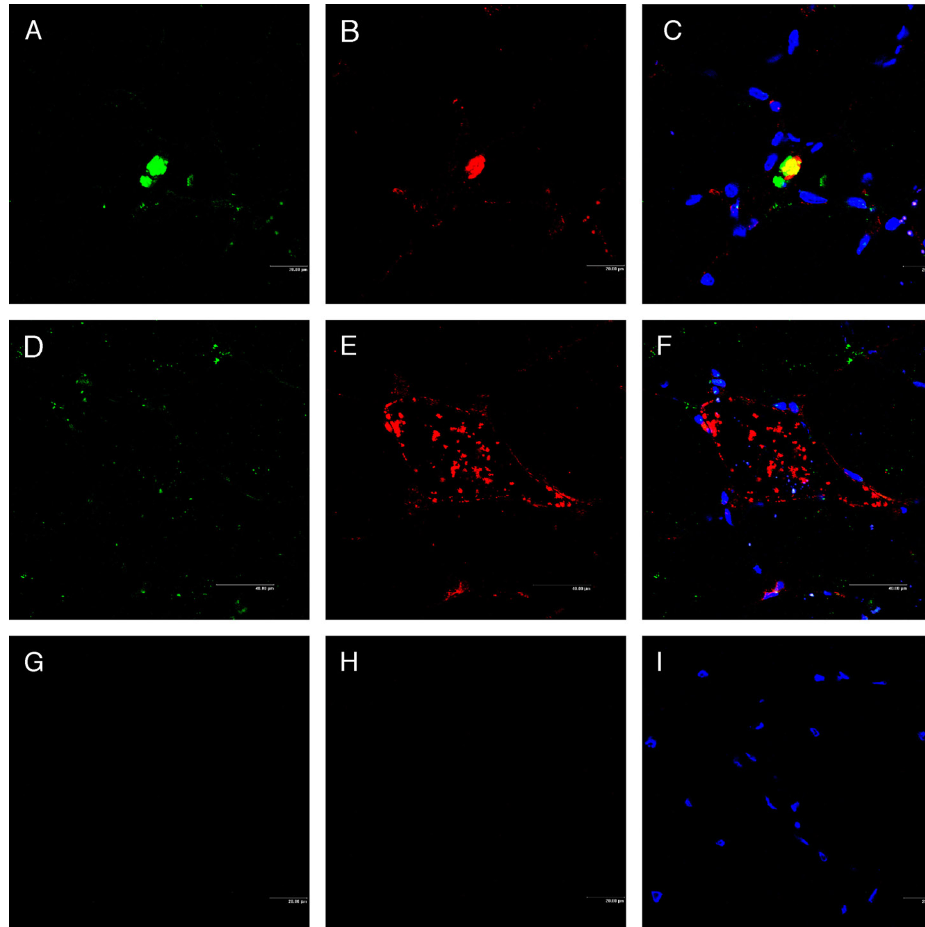

FIGURE 3. Double labeling immunofluorescence and confocal microscopy to ubiquitin **(**green, **A, D)** and TAR DNA-binding protein 43 **(**TDP-43; red, **B, E)** in myotilinopathy. Partial colocalization of ubiquitin and TDP-43 **(**merge, yellow, **C)** occurs in some fibers. Some round inclusions **(**small green dot in **A)** are not stained with TDP-43 antibodies **(**see **B** for comparison**)**, whereas some TDP-43 granular cytoplasmic deposits **(E)** are barely stained with anti-ubiquitin antibodies **(D)**, resulting in very little colocalization **(**merge, yellow **F)**. One section of the same case stained only with the secondary antibodies is used as a negative control **(G–I)**. Nuclei are visualized with To-pro-3-iodide (blue). Note decreased or absent TDP-43 immunoreactivity in the nuclei of fibers containing abnormal TDP-43 aggregates.

and in the biopsy of the patient who had IBMPFD (Fig. 1F). TAR DNA-binding protein 43–positive deposits occurred as single or several rounded well-defined intracytoplasmic inclusion bodies or as small multiple granular intracytoplasmic aggregates. Both types of inclusions occurred equally in sIBM and IBMPFD. Nuclear TDP-43 immunostaining was also reduced in damaged fibers in sIBM and IBMPFD.

No TDP-43 staining was seen in target lesions in samples from cases with denervation-reinnervation (data not shown). As in control cases, TDP-43 immunostaining in denervated fibers was restricted to the nuclei.

Double Labeling Immunofluorescence and Confocal Microscopy

Double labeling immunofluorescence in myotilinopathies showed that TDP-43 largely colocalized with myotilin (Fig. 2A–C), but TDP-43 colocalized less frequently with ubiquitin (Fig. 2D–F); only a few abnormal fibers were immunostained equally with anti-ubiquitin and anti–TDP-43 antibodies. Similarly, TDP-43 did not colocalize with desmin or with ubiquitin in most desminopathy cases (data not shown).

In sIBM samples, TDP-43 immunofluorescence colocalized with ubiquitin in many, but not all, round intracytoplasmic inclusions (Fig. 3A–C), but much more rarely in TDP-43–immunoreactive small granular aggregates (Fig. 3D–F). TAR DNA-binding protein 43 largely colocalized with valosin in cytoplasmic inclusion bodies in IBMPFD (Fig. 4A–C) but not in small aggregates (Fig. 4D–F).

TAR DNA-binding protein 43 immunoreactivity was decreased or absent in the nuclei of fibers containing abnormal TDP-43–immunoreactive aggregates, as revealed by TDP-43 and To-pro-3-iodide staining (Figs. 2–4).

Phosphorylated TDP-43

In control muscle fibers, antibodies against phospho–TDP-43 were negative in both cytoplasm and nuclei. In contrast, antibodies to phospho–TDP-43 showed deposition in the cytoplasm of vulnerable fibers in myotilinopathy, desminopathy, sIBM, and IBMPFD. Similar deposits were observed

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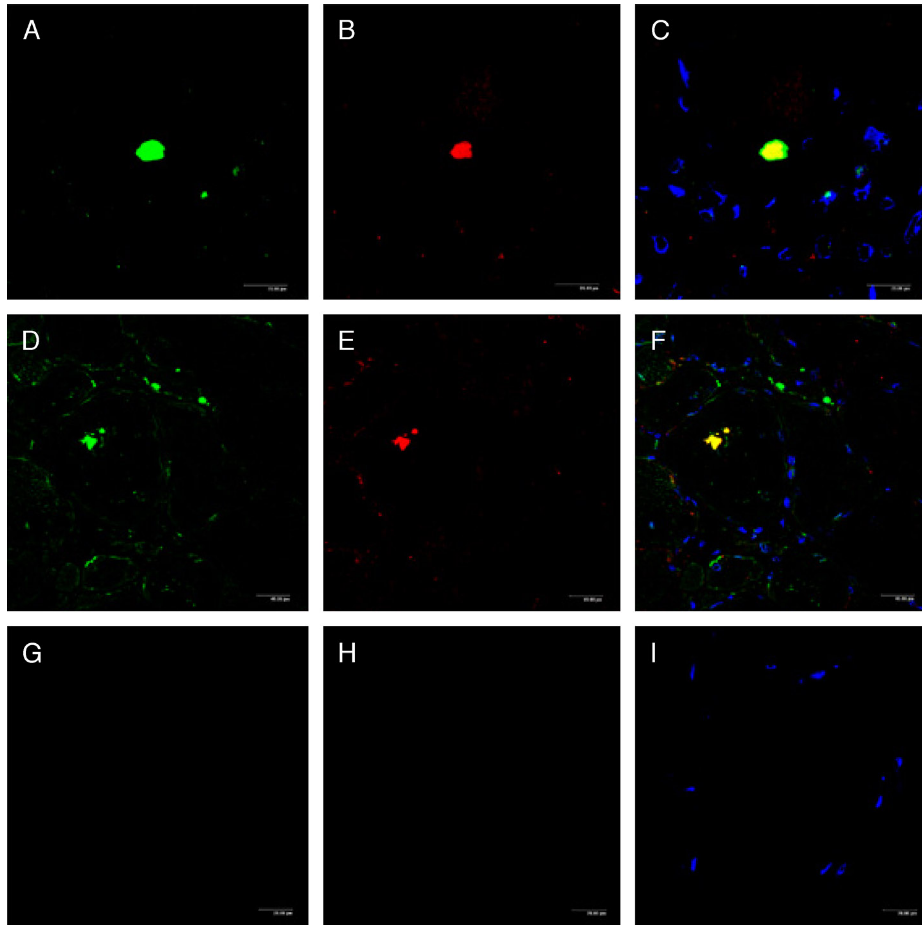

FIGURE 4. Double labeling immunofluorescence and confocal microscopy to TAR DNA-binding protein 43 (TDP-43; green, **A)** and valosin (red, **B)** show colocalization in inclusion cytoplasmic bodies in inclusion body myositis with Paget disease of bone and frontotemporal degeneration (IBMPFD; merge yellow, **C)**. However, ubiquitin (green, **D)** only occasionally colocalizes with TDP-43 (red, **E)** in small granular inclusions (yellow merge, **F)**. One section of the same case stained only with the secondary antibodies is used as a negative control (**G–I)**. Nuclei are visualized with To-pro-3-iodide (blue). Note the absence of TDP-43 immunoreactivity in the nuclei of fibers containing abnormal TDP-43 aggregates.

using the monoclonal and the polyclonal antibodies. The amount of aberrant deposition varied among cases (data not shown). To characterize phospho–TDP-43 deposition further, double labeling immunofluorescence and confocal microscopy disclosed partial colocalization of phospho–TDP-43 and TDP-43 in myotilinopathy and desminopathy (Fig. 5). Not all TDP-43–positive deposits were stained with anti-phospho–TDP-43 antibodies, and not all phospho–TDP-43–positive inclusions were recognized by anti–TDP-43 antibodies.

Similarly, double labeling immunofluorescence in sIBM (Fig. 6A–C) and IBMPFD (Fig. 6D-I) showed partial colocalization of phospho–TDP-43 and TDP-43 in abnormal protein inclusions. This was particularly clear in fibers with diffuse precipitates (Fig. 6G–I), whereas dense inclusions in IBMPFD were stained with anti–TDP-43 and phospho–TDP-43 antibodies equally (Fig. 6D–F).

Western Blotting

Western blots for TDP-43 revealed a band of approximately 43 kDa in myotilinopathies, desminopathies, sIBM, and control cases. The density of this band was significantly higher in myotilinopathies, desminopathies, and sIBM than in control samples ($p < 0.01$; Student *t*-test; Fig. 7A). Additional bands of lower molecular weight were observed in both pathologic and control cases (data not shown).

TDP-43 mRNA Expression Levels

GUS and B2M were appropriate endogenous controls to be used for normalization (averaged ΔCt value less than ± 0.5). No differences in TDP-43 mRNA expression levels were found in MFM and sIBM when compared with controls (Fig. 7B).

DISCUSSION

This study demonstrates prominent cytoplasmic accumulation of TDP-43 in damaged muscle fibers in myotilinopathy. TAR DNA-binding protein 43 colocalizes with myotilin and ubiquitin in some fibers but not in others; some fibers contain myotilin aggregates without accompanying TDP-43. Similarly, not all ubiquitinated inclusions colocalized with TDP-43, and, conversely, TDP-43 aggregates were

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found in some fibers with no ubiquitin deposition. Similar results were obtained in desminopathy, although cytoplasmic TDP-43–immunoreactive deposition was less prominent in desminopathy than in myotilinopathy. In contrast to normal fibers in which TDP-43 immunoreactivity localized in nuclei, reduced or absent TDP-43 immunoreactivity occurred in the nuclei of damaged fibers with TDP-43–immunoreactive cytoplasmic inclusions in MFMs.

While this article was in preparation, Weihl et al (33) reported TDP-43 accumulation in the sarcoplasm of affected muscles in IBMPFD and in sIBM. Muscle fibers in sIBM showed small cytoplasmic TDP-43 aggregates, whereas muscle biopsies from IBMPFD patients showed large peripherally located TDP-43–positive inclusions, suggesting a distinct pattern of TDP-43 immunostaining in sIBM compared with IBMPFD. Our results confirm that TDP-43 is a component of the inclusions in sIBM and IBMPFD. However, we observed both types of TDP-43–positive inclusions (i.e. small granular aggregates and large inclusion bodies) equally in all sIBM cases and in the only IBMPFD sample examined. As in MFMs, TDP-43 was reduced or absent in the nuclei of affected myofibers. TAR DNA-binding protein 43 largely colocalized

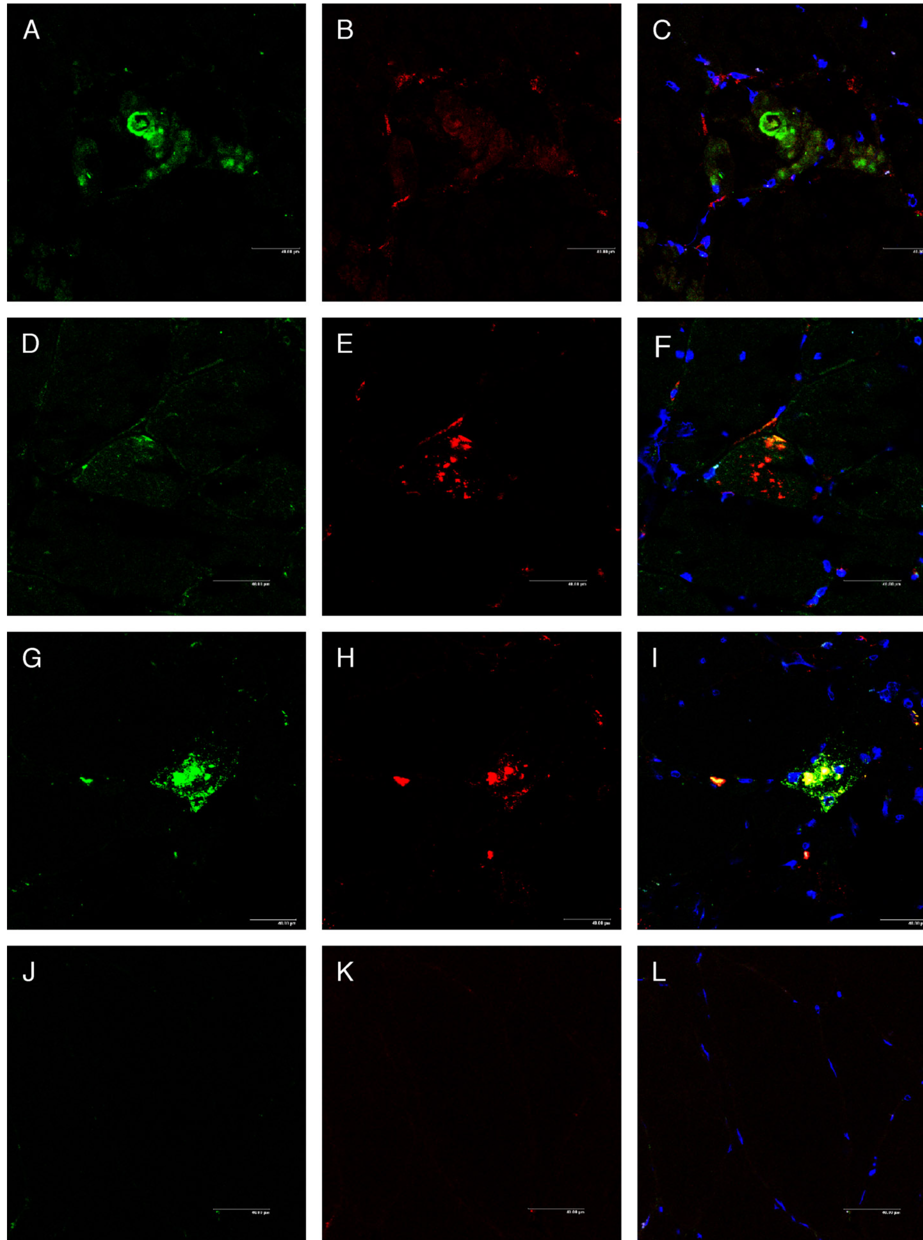

FIGURE 5. Double labeling immunofluorescence and confocal microscopy to phospho–TAR DNA-binding protein 43 (TDP-43; **A, D, G**, green) and anti–TDP-43 (**B, E, H**, red) in myotilinopathy (**A–C**) and desminopathy (**D–I**). Only partial colocalization of phospho–TDP-43 and TDP-43 is found in abnormal protein aggregates (**C, F, I**, merge, yellow). One section of the same case stained only with the secondary antibodies is used as a negative control (**J–L**). Nuclei are visualized with To-pro-3-iodide (blue).

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with ubiquitin in the large inclusion bodies in sIBM and IBMPFD but not in the small aggregates. As in myotilinopathies and desminopathies, TDP-43 was not always ubiquitinated in sIBM and IBMPFD. This is not surprising because TDP-43 has been found not to be the major target for ubiquitination in ALS, and TDP-43 immunoreactivity does not always colocalize with ubiquitin in skein-like inclusions in ALS (36).

In agreement with the immunohistochemical findings, increased TDP-43 expression levels, as revealed in Western blots, were found in myotilinopathies, desminopathies, sIBM, and IBMPFD. Interestingly, a smear of high molecular weight, which is observed in TDP-43 proteinopathies (22–25), was also observed in all diseased samples but not in control cases. We do not know whether this corresponds to ubiquitinated or phosphorylated TDP-43 at present. Increased protein levels of TDP-43 in myotilinopathies, desminopathies, and sIBM were not accompanied by upregulation of the *TARBBP* gene. Therefore, increased TDP-43 protein levels and TDP-43

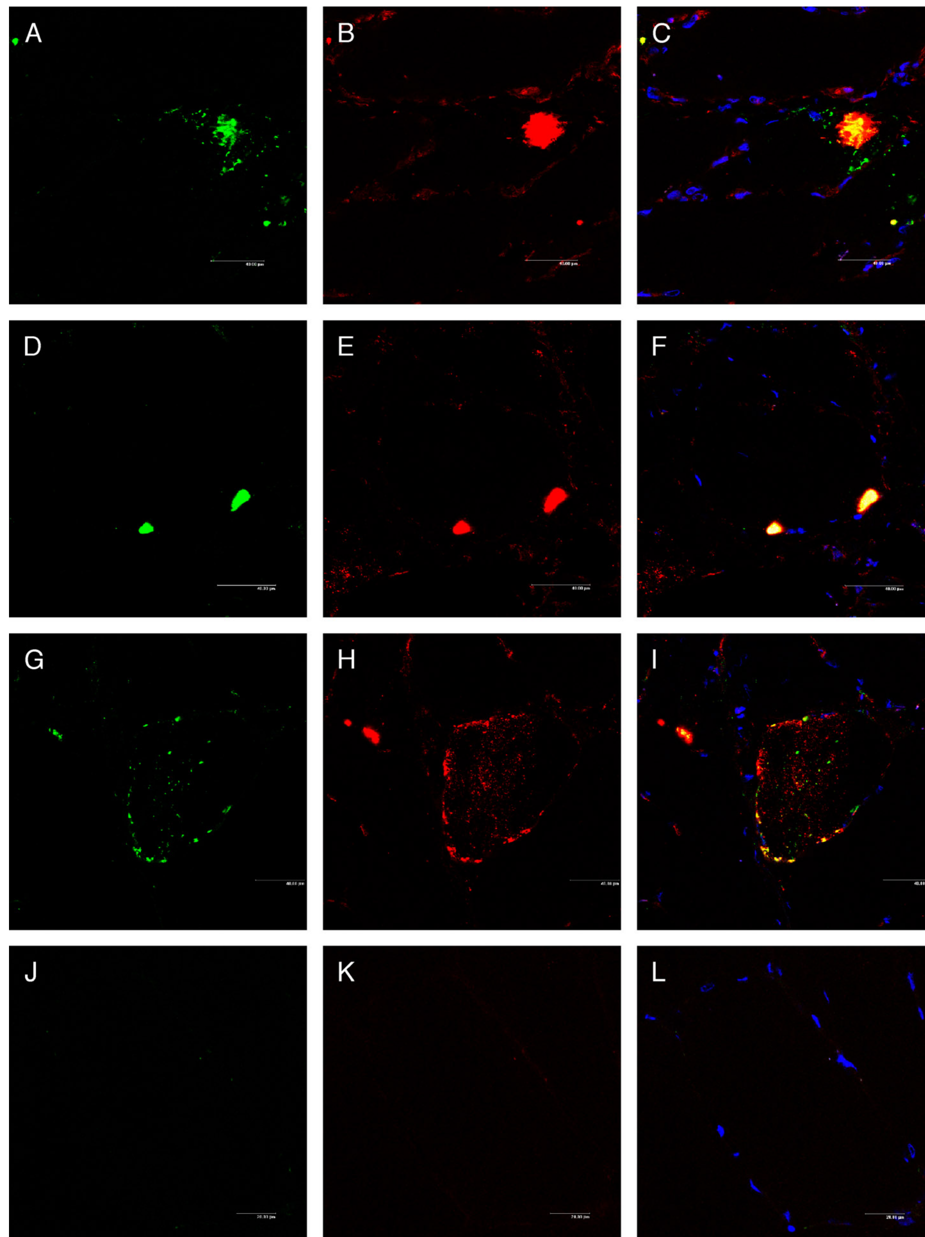

FIGURE 6. Double labeling immunofluorescence and confocal microscopy to phospho–TAR DNA-binding protein 43 **(**TDP-43; **A, D, G**, green**)** and anti–TDP-43 **(B, E, H**, red**)** in sporadic inclusion body myositis **(A–C)** and inclusion body myositis with Paget disease of bone and frontotemporal degeneration **(**IBMPFD; **D–I)**. Partial colocalization of phospho–TDP-43 and TDP-43 is seen in most deposits except the dense inclusions in IBMPFD **(D–E)**, as seen in corresponding merge images **(C, F, I**, yellow**)**. One section of the same case stained only with the secondary antibodies is used as a negative control **(J–L)**. Nuclei are visualized with To-pro-3-iodide (blue).

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aggregates in MFMs and sIBM are not the result of increased protein synthesis.

Modifications in TDP-43 immunoreactivity were not seen in target fibers in denervation atrophy cases, thus indicating selective involvement of TDP-43 in PAM.

The presence of TDP-43–positive inclusions in nerve and glial cells is consistently found in many cases of FTLD with ubiquitin-immunoreactive inclusions, FTLD with motor neuron disease, and ALS (22–24, 37–39). TAR DNA-binding protein 43 mutations have been reported in familial and sporadic ALS (40–42). For this reason, it has been deduced that all of these disorders are within the same disease spectrum, which has been designated "TDP-43 proteinopathy" (25, 43). The number of neurologic disorders associated with pathologic aggregates of TDP-43 has increased, however, and TDP-43 pathology has been described in Guam ALS and parkinsonism-dementia complex, a subset of α-synucleinopathies and tauopathies, as well as in hippocampal sclerosis (39, 44–47). TAR DNA-binding protein 43 pathology has not been found in association with anoxia, neoplasia, or ischemia, but TDP-43 immunoreactivity is present in Rosenthal fibers and eosinophilic granular bodies (48). Abnormal TDP-43 distribution and localization in such varied and unrelated degenerative diseases of the nervous system, together with its aberrant accumulation in muscle in genetic diseases caused by mutations in *MYOT*, *DES*, and *VCP*, as well as in sporadic IBM cases, makes it unlikely that there is a common TDP-43 origin for these disorders.

As a transcription factor, active TDP-43 is primarily located in the nuclei, but in FTLD-U, ALS, and PMA, TDP-43 is sequestered in intracytoplasmic inclusions with associated reduced detection of nuclear TDP-43 by immunohistochemical methods. The reasons for cytoplasmic accumulation with reduced nuclear localization in affected fibers are not known. Two theories have been advanced: one of them indicates extrusion of TDP-43 from the nuclei; the other postulates abnormal transport from the cytoplasm where TDP-43 is produced to the nucleus. Whatever the reason, 2 different conclusions are suggested by these data. On one hand, cytoplasmic TDP-43 accumulation in MFMs may contribute to aggregate formation; on the other hand, reduced nuclear TDP-43 expression may impair gene processing.

The mechanisms leading to protein aggregation in muscle fibers in MFM and sIBM seem to be associated with impairment of the proteolytic function of the ubiquitin-proteasome protein system (10, 16, 49). Several factors, including mutant proteins, aggresome formation, mutant ubiquitin, and post-translational protein modifications such as phosphorylation, oxidation, and nitration, have been shown to play a role in MFMs (10, 35, 49–52). Recent studies have demonstrated that perturbation of endogenous TDP-43 trafficking between the nucleus and the cytoplasm leads to TDP-43 aggregation (53).

Finally, it is important to stress that accumulated TDP-43 in FTLD-U with and without motor neuron disease, as well as in sporadic and familial ALS, is phosphorylated (22, 23, 54, 55). The present study shows that phosphorylated TDP-43 is also accumulated in the cytoplasm in sIBM, IBMPFD, myotilinopathies, and desminopathies. Double labeling immunofluorescence and confocal microscopy disclosed, however, that there is not an exact colocalization of TDP-43 and

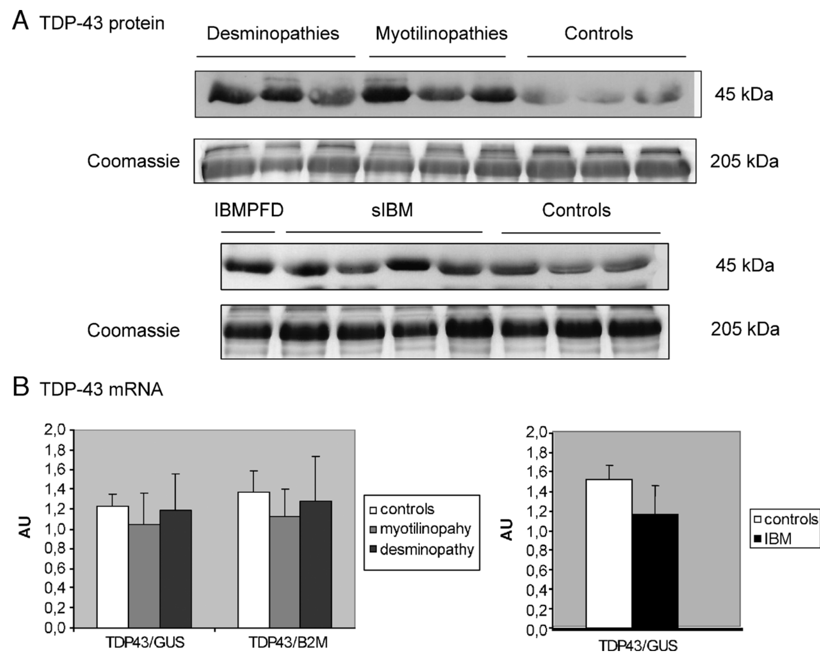

FIGURE 7. (A) Western blots to TAR DNA-binding protein 43 (TDP-43) in desminopathies, myotilinopathies, sporadic inclusion body myositis (sIBM), inclusion body myositis with Paget disease of bone and frontotemporal degeneration, and controls showing increased TDP-43 expression levels in diseased cases when compared with controls. Densitometric studies of all grouped cases showed significant differences between desminopathies, myotilinopathies, and sIBM when compared with controls ($p < 0.01$ in every group; not shown). **(B)** TAR DNA-binding protein 43 mRNA expression levels in myotilinopathies, desminopathies, and sIBM compared with controls. No differences in mRNA expression levels are seen between diseased cases and controls.

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phosphorylated TDP-43, thus indicating that not all TDP-43 accumulated in the cytoplasm of vulnerable fibers is modified by phosphorylation, at least, at Ser409/410 and Ser403/404 as revealed by specific anti–phosphor–TDP-43 antibodies. Therefore, increased TDP-43 phosphorylation in the cytoplasm further indicates that abnormal TDP-43 in PAM probably contributes to abnormal TDP-43 function, but phosphorylation of TDP-43 at those specific sites is not responsible for all the aberrant accumulation of TDP-43.

The functions of TDP-43 are not understood at present. It is involved in the control of gene transcription, transcription of selected splicing processes, and maintenance of mRNA stability (19). TAR DNA-binding protein 43 has been implicated in the promotion of mRNA stability of the human low molecular weight neurofilament (56), which may play a role in ALS (57). With respect to degenerative diseases of the nervous system and muscle, it is also important to stress that TDP-43 is associated with the Microprocessor complex Drosha/DGR8 (58), a major complex in the processing of microRNAs, which in turn regulates gene expression (59–61). Recent studies have demonstrated the crucial role of certain microRNAs in the development, physiology, and disease of striated muscle (62–66). Moreover, microRNA-206 seems to be uniquely expressed in muscle (67). It is also known that myogenic regulators regulate the expression of certain microRNAs (microRNA-1 and microRNA-206) in the chick embryo (68). Therefore, it seems clear that TDP-43 is a very robust candidate for the control of gene transcription in muscle. Whether this is the case, loss of TDP-43 in the nuclei and its aberrant accumulation in the cytoplasm of damaged fibers may disrupt part of the microRNA machinery controlling gene transcription in sIBM, IBMPFD, myotilinopathies, and desminopathies.

ACKNOWLEDGMENTS

The authors thank Dr. O. Soto for the clinical study of the IBMPFD patient; Dr. P. Camaño, Dr. A. López de Munain, Dr. J. Armstrong, Dr. L.G. Goldfarb, and Dr. A. Shatunov for genetic studies; and T. Yohanann for editorial advice.

REFERENCES

1. Sherman MY, Goldberg Al. Cellular defenses against unfolded proteins: A cell biologist thinks about neurodegenerative disease. Neuron 2001; 29:15–32
2. Ellis RJ, Pinheiro TJT. Danger-misfolded proteins. Nature 2002;416: 483–84
3. Carrell RW, Lomas DA. Conformational disease. Lancet 1997;350: 134–38
4. de Pril R, Fischer DF, van Leeuwen FW. Conformational diseases: An umbrella for various neurological disorders with an impaired ubiquitin-proteasome system. Neurobiol Aging 2006;27:515–23.
5. Goebel HH, Muller HD. Protein aggregate myopathies. Semin Pediatr Neurol 2006;13:96–103
6. Goebel HH, Fardeau M, Olivé M, et al. 156th ENMC International Workshop: Desmin and protein aggregate myopathies, 9-11 November 2007, Naarden, The Netherlands. Neuromuscul Disord 2008;18: 583–92
7. Nakano S, Engel AG, Waclawik AJ, et al. Myofibrillar myopathy with abnormal foci of desmin positivity. I. Light and electron microscopy analysis of 10 cases. J Neuropathol Exp Neurol 1996; 55:549–62
8. De Bleecker JL, Engel AG, Ertl B. Myofibrillar myopathy with foci of desmin positivity. II. Immunocytochemical analysis reveals accumulation of multiple other proteins. J Neuropathol Exp Neurol 1996;55: 563–77
9. Selcen D, Ohno K, Engel AG. Myofibrillar myopathy: Clinical, morphological and genetic studies in 63 patients. Brain 2004;127: 439–51
10. Ferrer I, Olivé M. Molecular pathology of myofibrillar myopathies. Expert Rev Mol Med 2008;10:25
11. Selcen D, Engel AG. Mutations in myotilin cause myofibrillar myopathy. Neurology 2004;62:1363–71
12. Olive M, Goldfarb LG, Shatunov A, et al. Myotilinopathy: Refining the clinical and myopathological phenotype. Brain 2005;128:2315–26
13. Goldfarb LG, Park KY, Cervenáková L, et al. Missense mutations in desmin associated with familial cardiac and skeletal myopathy. Nat Genet 1998;19:402–3
14. Muñoz-Mármol AM, Strasser G, Isamat M, et al. A dysfunctional desmin mutation in a patient with severe generalised myopathy. Proc Natl Acad Sci U S A 1998;95:11312–17
15. Barrachina M, Moreno J, Juvés S, et al. Abnormal up-regulation of neuron-restrictive silencer factor target genes in human myotilinopathy. Am J Pathol 2007;171:1312–23
16. Askanas V, Engel WK. Inclusion-body myositis: A myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. Neurology 2006;66:S39–48.
17. Dalakas MC. Sporadic inclusion body myositis-diagnosis, pathogenesis and therapeutic strategies. Nat Clin Pract Neurol 2006;2:437–47
18. Ou SH, Wu F, Harrich D, et al. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J Virol 1995;69:3584–96
19. Buratti E, Baralle FE. Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. J Biol Chem 2001;276:36337–43
20. Buratti E, Brindisi A, Giombi M, et al. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: An important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. J Biol Chem 2005;280:37572–84
21. Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front Biosci 2008;13: 867–78
22. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006;314:130–33
23. Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006;351:602–11
24. Mackenzie IR, Bigio EH, Ince PG, et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann Neurol 2007;61:427–34
25. Neumann M, Kwong LK, Sampathu DM, et al. TDP-43 proteinopathy in frontotemporal lobar degeneration and amyotrophic lateral sclerosis: Protein misfolding diseases without amyloidosis. Arch Neurol 2007;64: 1388–94
26. Cruts M, Gijselinck I, van der Zee J, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006;442:920–24
27. Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006;442:916–19
28. Watts GD, Wymer J, Kovach MJ, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 2004;36: 377–81
29. Watts GD, Thomasova D, Ramdeen SK, et al. Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. Clin Genet 2007;72:420–26
30. Kimonis VE, Mehta SG, Fulchiero EC, et al. Clinical studies in familial VCP myopathy associated with Paget disease of bone and frontotemporal dementia. Am J Med Genet 2008;146A:745–57
31. Hübbers CU, Clemen CS, Kesper K, et al. Pathological consequences of VCP mutations on human striated muscle. Brain 2007;130:381–93

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32. Neumann M, Mackenzie IR, Cairns NJ, et al. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. J Neuropathol Exp Neurol 2007;66:152–57
33. Weihl CC, Temiz P, Miller SE, et al. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. J Neurol Neurosurg Psychiat 2008;79:1186–89
34. Olivé M, Armstrong J, Miralles F, et al. Phenotypic patterns of desminopathy associated with three novel mutations in the desmin gene. Neuromusc Disord 2007;17:443–50
35. Olivé M, van Leeuwen FW, Janué A, et al. Expression of mutant ubiquitin (UBB+1) and p62 in myotilinopathies and desminopathies. Neuropathol Appl Neurobiol 2008;34:76–87
36. Sanelli T, Xiao S, Horne P, et al. Evidence that TDP-43 is not the major ubiquitinated target within the pathological inclusions of amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2007;66:1147–53
37. Dickson DW, Josephs KA, Amador-Ortiz C. TDP-43 in differential diagnosis of motor neuron disorders. Acta Neuropathol 2007;114:71–79
38. Fujita Y, Mizuno Y, Takamata M, Okamoto K. Anterior horn cells with abnormal TDP-43 immunoreactivities show fragmentation of the Golgi apparatus in ALS. J Neurol Sci 2008;269:30–34
39. Nishihara Y, Tan CF, Onodera O, et al. Sporadic amyotrophic lateral sclerosis: Two pathological patterns shown by analysis of distribution of TDP-43–immunoreactive neuronal and glial cytoplasmic inclusions. Acta Neuropathol 2008;116;169–82
40. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008;319:1668–72
41. Kabashi E, Valdmanis PN, Dion P, et al. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat Genet 2008;40:572–74
42. Gitcho MA, Baloh RH, Chakraverty S, et al. TDP-43 A315T mutation in familial motor neuron disease. Ann Neurol 2008;63:435–38
43. Kwong LK, Uryu K, Trojanowski JQ, et al. TDP-43 proteinopathies: Neurodegenerative protein misfolding diseases without amyloidosis. Neurosignals 2008;16:41–51
44. Uryu K, Nakashima-Yasuda H, Forman MS, et al. Concomitant TAR–DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. J Neuropathol Exp Neurol 2008;67:555–64
45. Geser F, Wintion MJ, Kwong LK, et al. Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. Acta Neuropathol 2008;67:33–45
46. Higashi S, Iseki E, Yamamoto R, et al. Concurrence of TDP-43, tau and synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. Brain Res 2007;1184:284–94
47. Freeman SH, Spires-Jones T, Hyman BT, et al. TAR-DNA binding protein 43 in Pick disease. J Neuropathol Exp Neurol 2007;67:62–67
48. Lee EB, Lee VM, Trojanowski JQ, et al. TDP-43 immunoreactivity in anoxic, ischemic and neoplastic lesions of the central nervous system. Acta Neuropathol. 2008;115:305–11
49. Ferrer I, Martín B, Castaño JG, et al. Proteasomal expression, induction of immunoproteasome subunits, and local MHC class I presentation in myofibrillar myopathy and inclusion body myositis. J Neuropath Exp Neurol 2004;63:484–98
50. Ferrer I, Carmona M, Blanco R, et al. Involvement of clusterin and the aggresome in abnormal protein deposits in myofibrillar myopathies and inclusion body myositis. Brain Pathol 2005;15:101–8
51. Janué A, Odena MA, Oliveira E, et al. Desmin is oxidized and nitrated in affected muscles in myotilinopathies and desminopathies. J Neuropath Exp Neurol 2007;66:711–23
52. Janué A, Olivé M, Ferrer I. Oxidative stress in desminopathies and myotilinopathies: A link between oxidative damage and abnormal protein aggregation. Brain Pathol 2007;17:377–88
53. Winton MJ, Igaz LM, Wong MM, et al. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. J Biol Chem 2008;283:13302–9
54. Hasegawa M, Arai T, Nonaka T, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol 2008;64:60–70
55. Inukai Y, Nonaka T, Yoshida M, et al. Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. FEBS Lett 2008;582: 2899–904
56. Strong MJ, Volkening K, Hammond R, et al. TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. Mol Cell Neurosci 2007;35:320–27
57. Ge WW, Leystra-Lantz C, Wen W, Strong MJ. Selective loss of rans-acting instability determinants of neurofilament mRNA in amyotrophic lateral sclerosis spinal cord. J Biol Chem 2003;278: 26558–63
58. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004;432:235–40
59. Shivdasani RA. MicroRNAs: Regulators of gene expression and cell differentiation. Blood 2006;108:3636–53
60. Nelson PT, Keller JN. RNA in brain disease: No longer just "the messenger in the middle". J Neuropathol Exp Neurol 2007;66:461–8
61. Thum T, Cataluci D, Bauersachs J. MicroRNAs: Novel regulators in cardiac development and disease. Cardiovasc Res 2008;79: 562–70
62. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. J Appl Physiol 2007; 102:306–13
63. Zhao Y, Ransom JF, Li A, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 2007;129: 303–17
64. McCarthy JJ, Esser KA, Andrade FH. MicroRNA-206 is overexpressed in the diaphragm but not the hindlimb muscle of mdx mouse. Am J Physiol Cell Physiol 2007;293:C451–7
65. Callis TE, Deng Z, Chen JF, Wang DZ. Muscling through the microRNA world. Exp Biol Med 2008;233:131–38
66. van Rooij E, Liu N, Olson EN. MicroRNAs flex their muscles. Trends Genet 2008;24:159–66
67. McCarthy JJ. MicroRNA-206: The skeletal muscle-specific myomir. Biochem Biophys Acta 2008;1779:682–91
68. Sweetman D, Goljanek K, Rathjen T, et al. Specific requirements of MRFs for the expression of muscle specific microRNAs, mir-1, miur-206 and miR-133. Dev Biol 2008;321:491–99

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