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Research Paper

Muscle-dominant wild-type TDP-43 expression induces myopathological changes featuring tubular aggregates and TDP-43-positive inclusions

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ABSTRACT

Muscle histology of sporadic inclusion body myositis (sIBM) demonstrates inflammatory findings and degenerative features including accumulation of TAR DNA-binding protein of 43 kDa (TDP-43). However, whether sarcoplasmic accumulation of TDP-43 is a primary trigger of muscle degeneration or a secondary event resulting from muscle degeneration in the pathophysiology of sIBM remained unclear. Our study aimed to discover whether muscle-dominant expression of TDP-43 is a primary cause of muscle degeneration. We generated several lines of wild-type TDP-43 transgenic mice driven by a creatine kinase 8 promoter, and analyzed the phenotypes via biochemical, histological, and proteomic techniques. The mice showed increased serum levels of myogenic enzymes. Muscle histology demonstrated myopathic changes including fiber size variation, abundant tubular aggregates, and TDP-43 aggregation with upregulation of endoplasmic reticulum (ER) stress. Proteomic analysis with aggregated materials in degenerative myofibers identified increased sarcoplasmic reticulum (SR)/ER-resident proteins that regulated calcium homeostasis, as well as cytosolic 5′-nucleotidase 1A. Muscle-dominant wild-type TDP-43 expression indeed caused myotoxicity featuring tubular aggregates and TDP-43-positive inclusions.

Our observation suggested that TDP-43 aggregates might not be sufficient to trigger the pathogenesis of sIBM although myofiber sarcoplasmic aggregation of TDP-43 led to myofiber degeneration via ER stress and possibly calcium dysregulation, independently of inflammatory process.

1. Introduction

Sporadic inclusion body myositis (sIBM) is one of the most common inflammatory myopathies among aged populations in Western countries and Japan (Dalakas, 2006; Hori et al., 2014; Nakanishi et al., 2013; Suzuki et al., 2012). Patients with sIBM present with progressive muscle weakness and wasting. Thus far, no effective treatment has been developed, and patients become wheelchair dependent within several

Abbreviations: AST, aspartate aminotransferase; Aβ, amyloid β; ALS, amyotrophic lateral sclerosis; BiP, immunoglobulin heavy chain-binding protein; CK, creatine kinase; DAPI, 4′,6-diamidino-2-phenylindole; ER, endoplasmic reticulum; FTLD, frontotemporal lobar degeneration; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, hematoxylin-eosin; LDH, lactate dehydrogenase; mGT, modified Gomori trichrome; MTS, 3-(4,5-dimethylthiazol-2-yl)- 5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium; NT5C1A, cytosolic 5′-nucleotidase 1A; PCR, polymerase chain reaction; RV, rimmed vacuole; sIBM, sporadic inclusion body myositis; SR, sarcoplasmic reticulum; TDP-43, TAR DNA-binding protein of 43 kDa; UPS, ubiquitin proteasome

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years after sIBM onset (Benveniste et al., 2011; Cox et al., 2011; Peng et al., 2000).

Muscle histology of sIBM cases has demonstrated inflammatory findings such as mononuclear cell infiltrates surrounding non-necrotic myofibers, as well as degenerative features including myofibers with rimmed vacuoles (RVs) (Dalakas, 2011). Abnormal sarcoplasmic aggregation, including that of amyloid β (Aβ) and its oligomers and that of phosphorylated tau in the form of paired helical filaments, has been observed in muscle fibers from sIBM patients (Askanas and Engel, 2011). Congophilic aggregates, Aβ immunoreactivity, and SMI-31 immunoreactivity were suggested as histological hallmarks of sIBM muscles. TAR DNA-binding protein of 43 kDa (TDP-43) was shown to accumulate in degenerative myofibers of sIBM (Salajegheh et al., 2009; Weihl et al., 2008; Yamashita et al., 2013). TDP-43 is a major component of ubiquitinated inclusions involved in the pathology of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (Arai et al., 2006; Neumann et al., 2006). Salajegheh et al. (Salajegheh et al., 2009) found sarcoplasmic immunoreactivity for TDP-43 in 23% of myofibers from sIBM patients, whereas other markers, such as RVs, congophilic materials, SMI-31 immunoreactivity, and Aβ immunoreactivity, were detected less frequently. However, whether accumulation of TDP-43 in sIBM myofibers is a primary trigger of muscle degeneration or a secondary event resulting from muscle degeneration in the pathophysiology of sIBM remained unclear.

Certain studies found autoantibodies against cytosolic 5′-nucleotidase 1A (NT5C1A), which is highly specific to sIBM among muscle diseases, in serum samples from some patients with sIBM (Larman et al., 2013; Pluk et al., 2013). NT5C1A was shown to form aggregations especially in the perinuclear region or on RVs of affected myofibers in sIBM patients. Although we recently reported that anti-NT5C1A autoantibodies may affect protein degradation in myofibers in vitro and in vivo passive immunization model (Tawara et al., 2017), the pathogenic role of autoantibodies against NT5C1A in sIBM has remained unclear.

In the previous study, the neuron-specific overexpression of human wild-type TDP-43 caused the abnormal histological phenotypes with intracytoplasmic and intranuclear aggregates of TDP-43 in the gene dosage-dependent manner (Shan et al., 2010; Wils et al., 2010). Therefore, we hypothesized that intramuscular overexpression of TDP-43 may cause TDP-43 aggregates in the muscle fibers. Our study aimed to clarify whether muscle-dominant overexpression of TDP-43 would cause muscle degeneration. We also investigated the mechanism by which TDP-43 overexpression would cause muscle degeneration without relation to the inflammatory process.

2. Materials and methods

2.1. Cell culture, transfection, and cell viability

C2C12 cells were supplied by Riken Bioresource Center (Tsukuba, Japan). For transient transfection, cells were transfected with pCMVTag1-TDP-43-myc vector using Lipofectamine 2000 reagent (Life Technologies, Carlsbad, CA, USA). Twenty-four, 48, and 72 h after transfection, the cells were analyzed by a tetrazolium-based colorimetric assay (3-(4,5-dimethylthiazol-2-yl)- 5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium (MTS) test (Promega)). For supplementation of denatured TDP-43 protein in culture media, recombinant human TDP-43 protein with GST tag at N-terminal (H00023435-P01, Abnova, Taipei, Taiwan) was treated with continuous agitation for a week at room temperature, or incubated with 4 M sodium acetate buffer (pH 4.0) for 24 h at 37 °C followed by neutralization with 2 M sodium hydroxide. TDP-43 aggregates (10 μg/ml, 2.0 μl) were mounted on formvar-coated nickel grids, stained with 0.2% uranyl acetate solution, and examined by a transmission electron microscope (H-7500; Hitachi, Tokyo, Japan). C2C12 cells were supplemented with 10 μg/ml of recombinant TDP-43 proteins (untreated, agitated, or acid-treated) for 24 h, and then analyzed by MTS test. Mouse anti-GST (1:500 dilution; Cell Signaling Technology, Beverly, MA, USA) and Alexa 488-conjugated anti-mouse IgG antibodies (1:200 dilution; Life Technologies Corporation, Carlsbad, CA, USA) were used for immunofluorescence study to evaluate the efficiency of uptake of recombinant TDP-43 proteins.

2.2. Construction of plasmids and transgenic mice

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Kumamoto University. All experimental procedures were conducted in compliance with Kumamoto University IACUC guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Experiments have been reported in accordance with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Human full-length TDP-43 cDNA tagged with C-terminal myc derived from pCMVTag1-TDP-43-myc vector was subcloned into pENTR4-ICAM_E vector, after which CSII-CK8-TDP43myc vector was generated by means of the LR clonase reaction with CSII-CK8-RfA vector (kindly provided by Prof. Chamberlain, Department of Neurology, Washington University, Seattle, WA, USA). The purified *Sna*B1-*Pme*I fragment derived from the CSII-CK8-TDP43myc vector was microinjected into 500 eggs of C57BL/6 mice. Genomic DNA was extracted via a tail biopsy sample. Genotype analysis of human TDP-43 was performed by means of PCR with the set of primers as follows: CKTDP43-F1, TGTCCCCCG CCAGCTAGACTC; and CKTDP43-R1, TGTAGCGAAGCCCACACGCC. Positive mice were designated founder mice (F0). These founder mice were backcrossed with wild-type mice (C57BL/6).

2.3. Biochemical analysis of serum from transgenic mice

Mice were deeply anesthetized with diethyl ether and euthanized at 18 months of age. Serum levels of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) were measured by colorimetric methods using an automatic biochemical analyzer (JCA-BM6070; Nihon Denshi Co, Ltd., Tokyo, Japan) with diagnostic kits.

2.4. Immunohistochemical analysis

Histological analyses were performed with fresh-frozen tissues from thigh muscles of the female mice. These fresh-frozen tissue sections were first studied by using routine histochemical techniques including HE and mGT staining. Next, muscle sections were fixed with 4% paraformaldehyde and blocked with 5% normal donkey serum and 0.1% Triton-X in PBS. The following primary antibodies were used: rabbit anti-TDP-43 (Cat#. 10,782–2-AP, 1:250 dilution; Proteintech, Chicago, IL, USA), mouse anti-TDP-43 (Cat#. 66,734–1-Ig, 1:500 dilution; Proteintech, Chicago, IL, USA), mouse anti-Myc (1:250 dilution; Medical and Biological Laboratories, Nagoya, Japan), mouse anti-BiP/GRP78 (1:250 dilution; BD Biosciences, San Jose, CA, USA), mouse anti-CHOP/GADD153 (1:250 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and rabbit anti-NT5C1A (1:500 dilution; Abcam, Cambridge, MA, USA). Immunolabelled proteins were visualized by using the avidin-biotin-peroxidase complex method with a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) or Alexa 488- or Alexa 546-conjugated secondary antibodies (1:200 dilution; Life Technologies Corporation). For immunostaining of Aβ40 and Aβ42, the Amyloid β-Protein Immunohistostain Kit (Wako, Osaka, Japan) was used. For immunofluorescence, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories) and examined by using confocal microscopy (FV1200; Olympus, Tokyo, Japan) or fluorescence microscopy (BZ-9000; Keyence, Osaka, Japan). RGB plot profiles were obtained by using ImageJ 1.50i software (National Institutes of Health).