2008

TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia

Conrad C. Weihl
Washington University School of Medicine in St. Louis

P. Temiz
Celal Bayar University School of Medicine

S. E. Miller
Washington University School of Medicine in St. Louis

G. Watts
Boston Children's Hospital

C. Smith
University of Kentucky College of Medicine

See next page for additional authors

Follow this and additional works at: http://digitalcommons.wustl.edu/icts_facpubs

Part of the Medicine and Health Sciences Commons

Recommended Citation
http://digitalcommons.wustl.edu/icts_facpubs/92

This Article is brought to you for free and open access by the Institute of Clinical and Translational Sciences at Digital Commons@Becker. It has been accepted for inclusion in ICTS Faculty Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.
TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia

C C Weihl, P Temiz, S E Miller, et al.

J Neurol Neurosurg Psychiatry 2008 79: 1186-1189
doi: 10.1136/jnnp.2007.131334

Updated information and services can be found at:
http://jnnp.bmj.com/content/79/10/1186.full.html

These include:

References
This article cites 17 articles, 7 of which can be accessed free at:
http://jnnp.bmj.com/content/79/10/1186.full.html#ref-list-1

Article cited in:
http://jnnp.bmj.com/content/79/10/1186.full.html#related-urls

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic collections
Articles on similar topics can be found in the following collections

- Immunology (including allergy) (44655 articles)
- Memory disorders (neurology) (2902 articles)
- Muscle disease (1354 articles)
- Neuromuscular disease (8493 articles)
- Memory disorders (psychiatry) (4449 articles)
- Musculoskeletal syndromes (17111 articles)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://journals.bmj.com/cgi/subscriptions
TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia

C C Weihl, P Temiz, S E Miller, G Watts, C Smith, M Forman, P I Hanson, V Kimonis, A Pestronk

ABSTRACT

TAR DNA binding protein-43 (TDP-43) is one component of the ubiquitinated inclusions (UBIs) in some frontotemporal dementias (FTD-U). One form of FTD-U, due to mutations in the valosin containing protein (VCP) gene, occurs with an inclusion body myopathy (IBM). Since IBM has TDP-43 in UBIs, we looked for TDP-43 inclusions in IBM muscle. In normal muscle, TDP-43 is present in nuclei. In IBM muscle, TDP-43 is additionally present as large inclusions within UBIs in muscle cytoplasm. TDP-43 inclusions were also found in 78% of sporadic inclusion body myositis (sIBM) muscles. In IBM and sIBM muscle, TDP-43 migrated with an additional band on immunoblot similar to that reported in FTD-U brains. This study adds sIBM and hereditary inclusion body myopathies to the growing list of TDP-43 positive inclusion diseases.

TAR DNA binding protein-43 (TDP-43) is one component of the ubiquitinated inclusions in the brains of patients with frontotemporal dementias (FTD-U) and amyotrophic lateral sclerosis (ALS). A phosphorylated form of TDP-43 was also identified to be more prevalent specifically in FTD-U tissue. More recently, missense mutations have been identified in TOP-43 that cosegregate with autosomal dominantly inherited ALS. Little is known about the function of TDP-43. It is a ubiquitously expressed, highly conserved nuclear protein that may be a transcription repressor or activator of exon skipping as well as a scaffold for the valosin containing protein (VCP) gene. Evidence of sarcoplasmic staining (fig 1A). In normal muscular dystrophy, TDP-43 has been identified in affected muscles including those with congenital disorders.

The pathogenesis of IBM and the more common sporadic inclusion body myositis (sIBM) is unknown but may also be due to UPS dysfunction. Affected muscle has UBIs that contain proteins, such as β-amyloid and phosphorylated tau, known to aggregate in CNS degenerative disorders. This has led to the suggestion that sIBM is related pathophysiologically to neurodegenerative diseases. It is not known whether TDP-43 is a component of the inclusions in IBM and sIBM muscle tissue. We evaluated the localisation of TDP-43 in normal, IBM and sIBM skeletal muscle tissue.

METHODS

Patients with sIBM had typical patterns of muscle weakness on physical examination, an abnormal EMG with myopathic motor units and spontaneous activity, and a muscle biopsy with myopathic changes, rimmed vacuoles within muscle fibres and endomysial inflammation with focal invasion of muscle fibres. Five patients with IBM and missense mutations in the p97/valosin containing protein (VCP) gene (four with R155H and one with N387H) were participants in the IRB approved study. All muscle biopsies were processed and evaluated in the Washington University Neuromuscular Laboratory. Cryostat sections of rapidly frozen muscle were processed for muscle histochemistry and immunocytochemistry in our standard fashion. The presence of vacuoles was evaluated via routine histochemical methods, such as haematoxylin and eosin or modified Gomori-trichrome stains. Immunocytochemistry for each antibody was performed on tissue from patients and compared with normal tissue controls processed simultaneously. Primary antibodies used in this study were directed against CD8 (clone M7103), phosphorylated neurofilaments or other phosphorylated epitopes (SMI-31; Covance, Berkeley, California, USA), FK2 antibody to ubiquitinated proteins (PW8810-0500; BioMol, Plymouth Meeting, Pennsylvania, USA), TDP-43 antibodies: rabbit polyclonal antibody (ProteinTech Antibody Group, Chicago, Illinois, USA) and mouse monoclonal antibody 2E2-D3 (Abnova, Taipei, Taiwan). Double labelling immunofluorescence was performed using Alexa Fluor 488 and 594 conjugated secondary antibodies (Molecular Probes; Eugene, Oregon, USA). Immunoperoxidase was performed as previously described using peroxidase conjugated secondary antibodies (Sigma, St Louis, Missouri, USA). Immunoperoxidase was performed as previously described using peroxidase conjugated secondary antibodies (Sigma, St Louis, Missouri, USA). The specificity of TDP-43 immunostaining was confirmed with two different commercial antibodies and incubation with secondary antibody alone (either fluorescent or peroxidase conjugated). Immunobots were performed as previously described.

RESULTS

In four normal muscle biopsies, TDP-43 was localised within scattered myonuclei with no evidence of sarcoplasmic staining (fig 1A). In
contrast, all IBMPFD muscle tissue had large peripherally based TDP-43 positive sarcoplasmic inclusions that did not localise to myonuclei (fig 1B, 1C). These inclusions consistently colocalised with FK2, an antibody that recognises ubiquitinated proteins (fig 1D) and in some cases with other proteins known to aggregate. SMI-31 binding was less prominent than TDP-43 in IBMPFD muscle tissue (fig 1E).

A distinctively different pattern of TDP-43 immunostaining was seen in 21 of 27 sIBM muscle. TDF-43 immunostained multiple small sarcoplasmic aggregates, most commonly in small angular muscle fibres (fig 2A). These inclusions did not colocalise with myonuclei. TDP-43 was also present in debris surrounding some rimmed vacuoles (fig 2A). The TDP-43 inclusions in sIBM were usually ubiquitin negative (fig 2B), but occasionally colocalised with FK2. TDP-43 also colocalised with T cells at sites of inflammatory infiltrates (fig 2C). In contrast with sIBM, TDP-43 positive inclusions were found in only 1 of 12 steroid responsive polymyositis patient biopsies.

Immunoblots of normal, IBMPFD and sIBM patient tissue with an antibody to TDP-43 demonstrated an increase in TDP-43 immunoreactivity present at 43 kDa as well as a higher migrating band similar to the phosphorylated form seen in FTD-U patient tissue (fig 2D).

DISCUSSION

Disruptions in the UPS may be associated with the pathogenesis of several degenerative disorders. In particular, IBM muscle and FTD-U brain have UBIs that contain aggregated proteins. However, the principal molecular constituents of the UBIs seen in these diseases have been incompletely defined. Recent studies have identified TDP-43 as a component of the UBIs in FTD-U, including IBMPFD, and ALS brain tissue. As IBMPFD muscle also has UBIs, we examined the localisation of TDP-43 in normal, sIBM and IBMPFD patient skeletal muscle. We found that TDP-43 localised to myonuclei in normal muscle but, in IBMPFD and sIBM muscle, TDP-43 was additionally present as sarcoplasmic inclusions. This is associated with an increase in
TDP-43 protein levels as well as a higher molecular weight band seen in sIBM and IBMPFD patient muscle tissue via immunoblot when compared with normal patient tissue. A similar higher molecular weight band was identified as phosphorylated TDP-43 in patients with FTD-U and ALS. Whether the band seen in IBMPFD and sIBM muscle tissue is the same or another post-translationally modified form (ie, ubiquitinated) is not known.

TDP-43 inclusions were present in 100% of IBMPFD and 78% of sIBM patient muscle biopsies, while 0% of normal muscle and 8% of steroid responsive polymyositis patient muscle biopsies had similar TDP-43 inclusions. This suggests that TDP-43 immunohistochemistry may be helpful in confirming the diagnosis of sIBM. Whether the band seen in IBMPFD and sIBM muscle tissue is the same or another post-translationally modified form (ie, ubiquitinated) is not known.

TDP-43 inclusions were present in 100% of IBMPFD and 78% of sIBM patient muscle biopsies, while 0% of normal muscle and 8% of steroid responsive polymyositis patient muscle biopsies had similar TDP-43 inclusions. This suggests that TDP-43 immunohistochemistry may be helpful in confirming the diagnosis of sIBM. Whether the band seen in IBMPFD and sIBM muscle tissue is the same or another post-translationally modified form (ie, ubiquitinated) is not known.

Our findings in skeletal muscle are similar to those found in CNS disease. TDP-43 inclusions in FTD-U and ALS are cytoplasmic, similar to the predominantly sarcoplasmic inclusions in IBM muscle. The presence of ubiquitinated TDP-43 in disparate tissues (ie, IBMFD muscle and brain) in which the only commonality is mutant VCP, suggests that VCP dysfunction may play a role in TDP-43 aggregation.

TDP-43 contains RNA binding motifs that may be associated with its function. Interestingly, another hereditary IBM, oculopharyngeal muscular dystrophy, is caused by mutations in another RNA binding protein, FABPN1. Mutant FABPN1 forms intranuclear inclusions that contain ubiquitin and polyadenylated miRNA. It is not known whether a similar process occurs with TDP-45 in sIBM and IBMPFD.

Diseases that develop TDP-43 inclusions include FTD-U, ALS, IBMFD and sIBM, suggesting that similar pathogenic
mechanisms may be present. Additional studies will be needed to further define the role of these proteins and their dysregulation in central nervous system and skeletal muscle tissue.

**Competing interests:** None.

**Ethics approval:** Obtained.

**REFERENCES**