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

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ABSTRACT

TAR DNA binding protein-43 (TDP-43) is one component of the ubiquitinated inclusions in some frontotemporal degenerative disorders (FTD-U). One form of FTD-U, due to mutations in the valosin containing protein (VCP) gene, occurs with an inclusion body myopathy (IBMPFD). Since IBMPFD brain has TDP-43 in inclusions, we looked for TDP-43 inclusions in IBMPFD muscle. In normal muscle, TDP-43 is present in nuclei. In IBMPFD muscle, TDP-43 is additionally present as large inclusions within Ubiquitinated protein (UBIs) in muscle cytoplasm. TDP-43 inclusions were also found in 78% of sporadic inclusion body myositis (sIBM) muscles. In IBMPFD 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.

RESULTS

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 inclusions that contain ubiquitinated and phosphorilated 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 IBMPFD and sIBM muscle tissue. We evaluated the localisation of TDP-43 in normal, IBMPFD and sIBM skeletal muscle tissue.
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 IBMFFD, 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

Figure 1 (A) Normal muscle immunostained with anti-TAR DNA binding protein-43 (TDP-43) antibody and counterstained with DAPI to allow visualisation of nuclei. The figure is an overlay of anti-TDP-43 and DAPI images. Arrows denote blue nuclei with TDP-43 (red dots) in scattered myonuclei. (B) Inclusion body myopathy, Paget’s disease of the bone and frontotemporal dementia (IBMFPD) tissue from patients immunostained with anti-TDP-43 (brown) and counterstained with Congo red to allow visualisation of nuclei and myofibres. Arrows denote large inclusions, some of which are peripherally based. (C) Overlay of anti-TDP-43 (orange) and DAPI (blue) of IBMFPD patient tissue. Note that large peripheral inclusions do not localise within nuclei (arrows). (D) IBMFPD patient tissue co-immunostained with anti-TDP-43 (red) and FK2 (green). Note that TDP-43 inclusions colocalise with FK2 (ubiquitinated proteins) (arrows). (E) IBMFPD patient tissue co-immunostained with anti-TDP-43 (red) and SMI-31, an antibody against phosphorylated tau epitopes (green). Note that some TDP-43 inclusions colocalise with SMI31 (closed arrows) and others do not (open arrows).
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. At present, the most reliable antibody marker for the diagnosis of sIBM is SMI-31 (an antibody against phosphorylated tau epitopes) which is not present in all sIBM biopsies and found in other diseased muscle tissue. For example, in our hands, SMI-51 positive aggregates are present in 66% of sIBM patients compared with 17% of steroid responsive polymyositis patients (unpublished observations).

It is notable that the TDP-43 immunostaining pattern was different when comparing sIBM and IBMPFD muscle. sIBM had small punctuate TDP-43 positive inclusions throughout the sarcoplasm that occasionally colocalised with ubiquitin. IBMPFD muscle had large peripherally based TDP-43 positive inclusions that always colocalised with ubiquitin. Both sIBM and IBMPFD patient muscle had evidence of a higher molecular weight TDP-43 species on immunoblot.

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, IBMPFD 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-45 inclusions include FTD-U, ALS, IBMPFD and sIBM, suggesting that similar pathogenic

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**Figure 2** (A) Sporadic inclusion body myositis (sIBM) tissue from patients, immunostained with anti-TAR DNA binding protein-43 (TDP-43) (brown) and counterstained with Congo red to allow visualisation of nuclei and myofibres. TDP-43 inclusions are small, in angular fibres and occasionally surround rimmed vacuoles. (B) Overlay of sIBM patient biopsy immunostained with TDP-43 (red) and FK2, for ubiquitinated proteins (green). TDP-43 inclusions do not colocalise with ubiquitin. (C) sIBM patient tissue co-immunostained with anti-TDP-43 (red) and anti-CD8 (green at focal sites of inflammation). Note the colocalisation of TDP-43 and CD8. This is in contrast with an adjacent fibre (arrow) with sarcoplasmic TDP-43 inclusions that does not co-localise with CD8. (D) Muscle tissue from sIBM, inclusion body myopathy, Paget’s disease of the bone and frontotemporal dementia (IBMPFD) and normal patient biopsies were homogenised and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The subsequent gel was transferred to nitrocellulose and immunoblotted with an antibody to TDP-43. Normal tissue has a discrete band at 43 kDa consistent with TDP-43, while sIBM and IBMPFD have a more prominent band and also a higher migrating band. Myosin is show as a loading control. All tissues are from flash frozen biopsies except the lane noted with an asterisk which is from autopsy muscle. All bands are from the same autoradiograph and moved for presentation purposes.
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.

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