Mislocalization of TDP-43 in the G93A mutant SOD1 transgenic mouse model of ALS

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Abstract

Previous evidence demonstrates that TAR DNA binding protein (TDP-43) mislocalization is a key pathological feature of amyotrophic lateral sclerosis (ALS). TDP-43 normally shows nuclear localization, but in CNS tissue from patients who died with ALS this protein mislocalizes to the cytoplasm. Disease specific TDP-43 species have also been reported to include hyperphosphorylated TDP-43, as well as a C-terminal fragment. Whether these abnormal TDP-43 features are present in patients with SOD1-related familial ALS (fALS), or in mutant SOD1 over-expressing transgenic mouse models of ALS remains controversial.

Here we investigate TDP-43 pathology in transgenic mice expressing the G93A mutant form of SOD1. In contrast to previous reports we observe redistribution of TDP-43 to the cytoplasm of motor neurons in end stage disease in mSOD mice. These data indicate that TDP-43 mislocalization and ubiquitination are present in end stage mSOD mice. However, we do not observe C-terminal TDP-43 fragments nor TDP-43 hyperphosphorylated species in these end stage mSOD mice. Our findings indicate that G93A mutant SOD1 transgenic mice recapitulate some key pathological, but not all biochemical hallmarks, of TDP-43 pathology previously observed in human ALS. These studies suggest motor neuron degeneration in the mutant SOD1 transgenic mice is associated with TDP-43 histopathology.

Keywords:
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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease selectively affecting motor neurons of the brainstem and spinal cord, as well as cortical regions. Approximately 5–10% of ALS cases are inherited (fALS), however, the cause of the remaining 90–95% of sporadic ALS (sALS) cases remains unknown. Mutations of superoxide dismutase 1 (SOD1) account for approximately 15–20% of fALS cases (1–2% of all ALS cases), whereas various other gene mutations account for only a small subset of the remaining fALS cases [9]. Nervous system tissue from patients who died with fALS and sALS has been extensively studied to gain insight into the pathological features of ALS. To study the pathogenesis and investigate the details of the progression of ALS, transgenic rodent models harboring fALS-associated mutations in human mutant SOD1 (mSOD) have been developed. These models have proven to be of significant value [8] and replicate several key features observed in both sALS and fALS including progressive loss of motor neurons, neurofilament aggregation, and the accumulation of cytoplasmic ubiquitinated inclusions (UBIs) within degenerating motor neurons [5,20].

Recently, two independent groups [1,14] have reported that TAR DNA binding protein (TDP-43), a nuclear DNA and RNA binding protein proposed to function as a regulator of transcription [16] and alternative splicing [21,13] is present in UBIs in sALS. Furthermore, a number of TDP-43 mutations have been reported in both sALS and fALS cases, suggesting that TDP-43 may play a causal role in the pathogenesis of ALS [19,22,23]. TDP-43 is normally localized to the nucleus, however, in CNS tissue from patients who died with ALS, TDP-43 is redistributed from the nucleus to the cytoplasm, where it appears to be distributed diffusely, or to aggregate as a component of UBIs [1,14]. Although studies of TDP-43 in human ALS cases are generally consistent [1,4,12,14], some aspects of the TDP-43 pathology remain controversial. For instance, Mackenzie et al. [11] suggest that abnormal localization of TDP-43 is present in most sALS and fALS cases but is absent in fALS caused by SOD1 mutations. In contrast, Robertson et al. [17] showed, in two fALS cases carrying SOD1 mutations, that there is mislocalization of TDP-43 to the cytoplasm as well as association with UBIs. Studies of some lines of mSOD mouse models have claimed that there is no TDP-43
Transgenic mice expressing human G93A mutant SOD1 were purchased from Jackson Laboratories (B6.Cg-Tg(SOD1-G93A)1Gur, stock # 004435) or bred locally with C57BL6 female mice. Mice were genotyped using PCR [6] and wild-type (WT) littermates were used as controls in these studies. Protocols governing the use of animals were approved by the Animal Care Review Committee of Simon Fraser University and were in compliance with guidelines published by the Canadian Council on Animal Care (CCAC). A minimum of 3 mice per group were used in these studies. We defined end stage for mSOD mice as the appearance of a set of behavioral markers including an inability to forage due to paralysis of the hind limbs and an inability of the mice to right themselves within 10 s of lateral recumbency. Mean survival time of the mSOD mice reaching end stage was 178 ± 14 days (mean ± S.E.M., n = 9). Animals were culled with CO2, perfused transcardially with PBS, and subsequently with a 4% solution of paraformaldehyde (PFA) in PBS. The spinal cords were dissected out and postfixed in 4% PFA, left overnight in a solution of 20% sucrose in PBS for cryoprotection, and subsequently embedded in Tissue-Tek O.C.T compound (Sakura, Zoeterwoude, Netherlands). Transverse lumbar spinal cord sections of 50 μm were cut using a Leica cryostat. Sections were treated with PBS containing 0.3% Triton X-100 (PBST) for permeabilization, following by blocking with 5% BSA and 10% NGS. Anti-TDP-43 rabbit polyclonal antibody (Proteintech, 10782-2-AP) was diluted at 1:500 in PBST and incubated with free floating cryo-sections overnight at 4 °C. For double labelling experiments, anti-NeuN mouse monoclonal antibody (Chemicon, MAB378), diluted at 1:1000, anti-ubiquitin mouse monoclonal antibody (Chemicon, MAB1510) diluted in 1:500 were used, and immunoreactivities of interest detected using appropriate Cy3- and FITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). Sections were also counterstained for neurons with 100 ng/ml DAPI (4′,6-diamidino-2-phenylindole, Vector Laboratories). Immunolabelled spinal cord sections were imaged and analyzed using a Leica DM4000B microscope and images captured using a Spot digital camera (DFC350FX, diagnostic Instruments, Sterling Heights, MI) and Leica Application Suite (LAS2.5.0 R1). Labelling of mSOD transgenic mouse tissue was compared to tissues from both mSOD and WT littermate mice (Fig. 2a), indicating no similarities between G93A mSOD mice and these human ALS cases [10].

Dystrophic neurites were present in spinal cords of mSOD mice just before use, to generate a solution containing 0.1 mg tissue/mL. The tissue was homogenized on ice and then the mixture was centrifuged at 17,900 × g for 15 min at 4 °C. The resulting supernatants were diluted with Laemmli sample buffer, boiled for 5 min, and separated using SDS-PAGE. After electrophoresis, proteins were transferred onto nitrocellulose membranes at 100 V for 1.5 h. Membranes were then blocked for 1 h with 1% BSA or gelatin dissolved in PBS containing 0.1% Tween 20 (PBS-T) and then incubated with the specified primary antibodies overnight at 4 °C. The membrane was washed with PBS-T and blocked for another 0.5 h after which the membranes were incubated with the appropriate HRP-conjugated secondary antibodies for 1 h. Membranes were then washed with PBS-T and immunoreactive proteins visualized using ECL reagents and film (Amersham Bioscience, Piscataway, NJ).

Frozen lumbar spinal cords were weighed and sequentially extracted as previously described with slight modifications [14]. In brief, tissues were extracted at 200 mg/ml in low salt (LS) buffer (10 mM Tris, pH 7.5, 5 mM EDTA, 1 mM DTT, 10% sucrose, and a cocktail of protease inhibitors) by sonicating twice for 20 s and then centrifuging the resulting mixture at 25,000 × g for 30 min at 4 °C. Pellets were sequentially extracted in high salt buffer containing Triton X (TX) (LS containing 1% Triton X-100 and 0.5 M NaCl), sarcosyl-containing buffer (SA) (LS containing 1% N-lauroyl sarcosine and 0.5 M NaCl) and urea-containing buffer (UR) (7 M urea, 2 M thiourea, 4% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), 30 mM Tris, pH 8.5), followed by Western blot analysis as described above.

We examined lumbar spinal cords from 12-week-old mSOD mice for NeuN and TDP-43 histopathology, screening all stained sections visually for abnormal neuronal TDP-43 localization (Fig. 1). NeuN immunolabelling of 12-week-old mSOD ventral horn grey matter showed many healthy neurons resembling age-matched WT mice (Fig. 1a and d). TDP-43 immunoreactivity was observed exclusively in the nucleus in tissues from both mSOD and WT mice of this age (Fig. 1b, c, e and f). To more comprehensively evaluate potential TDP-43 pathology, we examined end stage G93A mSOD mice. Strikingly, motor neurons in lumbar spinal cords from end stage mSOD mice (Fig. 1g–i), but not those of WT animals (Fig. 1j–l) of the same age, showed strong cytoplasmic labelling with anti-TDP-43 antibodies. Furthermore, some of these motoneurons having cytoplasmic TDP-43 immunoreactivity showed axonal TDP-43 immunoreactivity that was sometimes also accompanied by punctate nuclear TDP-43 staining (Fig. 1m). Within the ventral horn of lumbar spinal cord, some TDP-43 immunoreactivity appeared in dystrophic neurite-like structures as well as rounded inclusions resembling Lewy body-like hyaline inclusions (LBHIs) (Fig. 1h), both of which have been previously observed and used to define TDP-43 pathology in human ALS tissues [1,14]. In addition, some surviving motor neurons displaying normal nuclear TDP-43 immunoreactivity are observed adjacent to neurons exhibiting a pathological cytoplasmic distribution of TDP-43 (Fig. 1h).

Previous studies of spinal cord tissue from patients who died with ALS have shown that some TDP-43 immunoreactive inclusions also contain ubiquitin, and that TDP-43 immunoreactivity is associated with the periphery of the ubiquitin-containing inclusions [1,3]. It also has been reported that LBHIs are immunoreactive for TDP-43 and ubiquitin in neurons from a sALS patient [15]. To investigate the similarities between G93A mSOD mice and these human ALS cases we analyzed ubiquitin immunoreactivity and its relation to TDP-43-positive structures in end stage mice. Immunohistochemistry using a ubiquitin-specific monoclonal antibody revealed strong ubiquitin immunoreactivity most frequently associated with rounded TDP-43-positive structures (Fig. 1n–s), but this was not associated with other TDP-43-positive structures such as dystrophic neurites. Therefore, the TDP-43 histopathological features of end stage G93A mSOD mice recapitulate those observed in human ALS cases. These observations suggest TDP-43 redistribution and aggregation occurs in G93A mSOD mice. We therefore assessed whether abnormal TDP-43 species were present in these end stage mSOD mice using Western blotting. First we examined TDP-43 protein expression in spinal cord tissue by Western blot. Full-length TDP-43 and a lower migrating species were detected in lysates obtained from both mSOD and WT littermate mice (Fig. 2a), indicating no difference in expression levels of TDP-43 between mSOD mice and WT controls. The identity of the rapidly migrating species remains unknown. Secondly, spinal cords were sequentially extracted in buffers of increasing ionic and detergent strength, essentially as previously described, and these fractions were then analyzed by
Western blot. No differences in the abundance or solubility of TDP-43 in different fractions from end stage mSOD and age-matched WT samples could be detected. Furthermore, we observed no pathological ∼25 kDa species nor ∼45 kDa hyperphosphorylated species, which have been previously detected in human ALS (Fig. 2c).

Recent reports have shown that TDP-43 is mislocalized in affected neurons in CNS tissue from ALS patients, moving from normal localization in the nucleus to the cytoplasm. In addition to TDP-43 mislocalization, both a ∼25-kDa C-terminally cleaved fragment and a ∼45-kDa hyperphosphorylated TDP-43 species have been reported in urea-soluble protein extracts from ALS tissues [14]. In this work, we have investigated whether similar abnormalities in TDP-43 localization occur in transgenic mice expressing the G93A mutant form of SOD1. In contrast to a previous report on mSOD mice, we observed a redistribution of TDP-43 to the cytoplasm of motor neurons in mutant SOD1 transgenic mice. The differing
In brief, our findings suggest that the mutant SOD1 transgenic mice (G93A) do recapitulate some pathological features, but not all of the biochemical hallmarks, of TDP-43 pathology that have been observed in human ALS. These studies suggest that motor neuron degeneration occurring in mutant SOD1 transgenic mice is associated with TDP-43 histopathology. Given that TDP-43 pathology in G93A mSOD mice of advanced age does recapitulate features observed in human ALS cases, this animal model may be a valuable tool for studying the progression and pathophysiology of ALS and as a useful model to test potential TDP-43-directed therapeutics.

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