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Research Paper

Dendritic Homeostasis Disruption in a Novel Frontotemporal Dementia Mouse Model Expressing Cytoplasmic Fused in Sarcoma

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ABSTRACT

Cytoplasmic aggregation of fused in sarcoma (FUS) is detected in brain regions affected by amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), which compose the disease spectrum, FUS proteinopathy. To understand the pathomechanism of ALS-FTD-associated FUS, we examined the behavior and cellular properties of an ALS mouse model overexpressing FUS with nuclear localization signal deletion. Mutant FUS transgenic mice showed hyperactivity, social interactional deficits, and impaired fear memory retrieval, all of which are compatible with FTD phenotypes. Histological analyses showed decreased dendritic spine and synaptic density in the frontal cortex before neuronal loss. Examination of cultured cells confirmed that mutant but not wild-type FUS was associated with decreased dendritic growth, mRNA levels, and protein synthesis in dendrites. These data suggest that cytoplasmic FUS aggregates impair dendritic mRNA trafficking and translation, in turn leading to dendritic homeostasis disruption and the development of FTD phenotypes.

1. Introduction

The identification of DNA- and RNA-binding protein TAR DNA-binding protein (TDP-43) as a component of inclusions in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) has led to an ALS research breakthrough in 2006 (Arai et al., 2006; Neumann et al., 2006). Since then, it has been proposed that FTD and ALS constitute a novel disease spectrum sharing a common molecular basis (Ito and Suzuki, 2011). In 2009, mutations in the gene encoding the RNA-binding protein fused in sarcoma (FUS) (also known as translocated in liposarcoma [TLS]) were identified as the cause of familial ALS (ALS6) (Kwiatkowski et al., 2009; Vance et al., 2009). Subsequently, several investigators reported observing FUS-positive inclusions in FTD that was classified as basophilic inclusion body disease (BIBD) and neuronal intermediate filament inclusion disease (NIFID) (Munoz et al., 2009; Neumann et al., 2009b). Thus, it has been proposed that mutant-FUS–linked ALS, atypical frontotemporal lobar degeneration with ubiquitinated inclusions, BIBD, and NIFID also comprise an ALS-FTD disease spectrum: FUS proteinopathy. Thereafter, a growing number of studies identified many causative genes of ALS-FTD, such as *C9orf72 G4C2* repeat expansion and *hnRNPA2B1*, emphasizing the importance of this novel disease spectrum (Kim et al., 2013; Majounie et al., 2012).

In FUS, which plays roles in DNA repair, transcription, alternative splicing, translation, and RNA transport (Ito and Suzuki, 2011), ALS-linked mutations are enriched at the nuclear localization signal at the C-terminus. Cellular analysis reveals that the increased mislocalisation of mutant protein into the cytoplasm is negatively correlated with the age of disease onset (Dormann et al., 2010; Ito et al., 2010), which is similar to polyglutamine diseases. The impairment of the nuclear transport of FUS is therefore directly associated with neurodegeneration in ALS and FTD. Furthermore, a mutation in a truncated protein lacking a nuclear localization signal was identified to cause juvenile ALS with rapid disease progression and cognitive impairment (Zou et al., 2013), suggesting that impaired nucleocytoplasmic trafficking of FUS alone can lead to the ALS phenotype (Waibel et al., 2010).

A major unresolved question is whether FUS-mediated neurodegeneration is caused by a toxic gain of function by cytoplasmic FUS aggregates or a loss of normal FUS function in the nucleus. Recently, we generated a transgenic mouse line overexpressing exogenous FUS with nuclear localization signal deletion (ΔNLS-FUS), reflecting juvenile ALS (Shiihashi et al., 2016). This FUS transgenic (tg) mouse, in which exogenous FUS protein is located strictly in cytoplasm at moderate level (~80% of the endogenous FUS level), showed significant progressive motor impairment from 20 weeks of age. Pathological analysis revealed

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ubiquitin- and p62-positive cytoplasmic ΔNLS-FUS aggregates and also astrocytosis, microgliosis, and neuronal loss in the brains of FUS tg mice at 1 year, indicating the recapitulation of some aspects of pathological ALS. However, the expression level, nuclear distribution, and function of RNA splicing of the endogenous FUS were not changed in FUS tg mice. We therefore concluded that the toxic gain of function of cytoplasmic FUS aggregates was sufficient to lead to neurodegeneration (Shiihashi et al., 2016).

To understand the pathomechanism of cytoplasmic FUS aggregates in ALS-FTD, it is necessary to establish a novel FTD model of FUS proteinopathy. Several groups have reported that transgenic mice with mutant FUS showed ALS-like motor deficits (Qiu et al., 2014; Scekic-Zahirovic et al., 2017; Scekic-Zahirovic et al., 2016; Sharma et al., 2016); however, thus far none has evaluated FTD phenotypes. In this study, we examined the behavior and cognitive function of ΔNLS-FUS tg mice before the appearance of motor deficits. We also performed anatomical and cellular examinations in order to understand the molecular basis of neurodegeneration by cytoplasmic FUS aggregates. During behavioral analysis, FUS tg mice showed hyperactivity, social interactional deficits, and impaired fear memory retrieval, all of which are compatible with FTD phenotypes. Regarding the pathological mechanism of cytoplasmic aggregation of FUS, histological analysis revealed that cytoplasmic FUS aggregates recruited robust mRNA and RNA transporters such as Staufen and fragile X mental retardation protein (FMRP). Golgi staining and electrophysiology showed decreased dendritic spine density in the frontal cortex and hippocampus of FUS tg mice before neuronal loss. Moreover, examination of primary cortical cultured neurons confirmed that mutant but not wild-type FUS decreased dendritic growth, mRNA levels, and protein synthesis in dendrites. These data suggest that cytoplasmic FUS aggregates trap mRNA and its transporters, impairing dendritic mRNA trafficking and translation, in turn leading to the disruption of dendritic homeostasis and the development of FTD phenotypes.

2. Materials and Methods

2.1. Generation of ΔNLS-FUS Tg Mice

This procedure is described in full in the article of our previous study (Shiihashi et al., 2016); briefly, a human FUS cDNA with deleted NLS was inserted into the pTSC21k vector encoding the murine Thy1.2 expression cassette. Tg lines were bred and backcrossed more than five generations with C57Bl6/J mice. Mice were housed in standardized ventilated microisolation caging (four animals per cage). Genotyping from tail DNA was performed using the following primer pairs: 5′-AAGAAGACCTGGCCTCAAACG-3′ and 5′-TATCCCTGGGGAGTTGACTG-3′. Animal experiments were approved by the Committee on Animal Care and Use, Keio University (09217- [3]) and conducted according to the Animal Experimentation Guidelines of Keio University School of Medicine.

2.2. Behavioral Tests

Male non-tg ($n = 15$) and FUS tg ($n = 14$) mice between 9 and 13 weeks of age were used for the following behavioral tests, which were performed sequentially.

Home cage test: Each mouse (9 weeks old) was placed alone in a testing cage (18.8 cm [W] × 28.8 cm [L] × 13.7 mm [H]) under a 12-h light-dark cycle (light on at 08:00) and with free access to both food and water. Spontaneous activity in the cage was assessed for 3 continuous days by counting the number of infrared beam crossings (Scanet; Melquest).

Open-field test: Each mouse (11 weeks old) was placed in an open-field arena (50 cm [W] × 50 cm [L] × 40 cm [H]; O'Hara & Co., Ltd.) made of white polyvinyl chloride. The distance travelled in the open field was recorded for 30 min using a video-imaging system (Image OF9; O'Hara & Co., Ltd.). The central area was defined as the central 18 cm × 18 cm region of the arena. The mice were tested on two consecutive days.

Y-maze test: The Y-maze test was performed at 11 weeks of age after the open-field test. The apparatus consisted of a plastic maze with three V-shaped arms (40 cm [L] × 3 cm [W] at the bottom and 10 cm [W] at the top opening × 12 cm [H], 120 degrees' separation). Mice were allowed to move freely through the maze for 10 min. The sequence and total number of arm entries were recorded by a video camera. A "novel arm selection" was counted when a mouse successively entered three different arms. The recorded data were analyzed offline with EthoVision XT software (Noldus Information Technology).

Three-chamber test: We evaluated social recognition and response to social novelty using the three-chamber test at 12 weeks of age. The apparatus consisted of a white plastic box (42 cm [W] × 61.5 cm [L] × 22 cm [H]) with partitions dividing the box into three equal-sized chambers, with 10-cm openings between chambers. The side chamber has a plastic triangular prism cage at the distal corner for constraining a stranger mouse. Target subjects (stranger one and stranger two) were age-matched males. This task was carried out in three phases of 10 min each. In phase one, the test mouse was placed in the middle chamber and left to explore the area containing the empty cages for 10 min. In phase two, the mouse was placed in the middle chamber, but an unfamiliar mouse (stranger one) was placed into a cage in one of the side chambers. In phase three, a novel stranger mouse (stranger two) was placed in the previously empty cage and again the test mouse was left to explore for 10 min. We measured the time the test mouse spent in each chamber for each phase.

Trace fear conditioning test: This test was carried out in mice aged 13 weeks according to the procedure described previously, with minor modifications (Suh et al., 2011). On day one, the mouse was placed in a conditioning chamber. Each mouse was exposed to tone-foot shock pairing (tone = 20 s, 65 dB white noise; footshock = 2 s, 0.75 mA, 20 s after termination of tone). The tone-footshock pairing was performed three times (beginning at 240, 400, and 560 s). Mice were returned to their home cages 98 s after the last footshock. Fear memory of the tone was investigated on day two by placing each mouse in a novel chamber. After 4 min of free exploration, each mouse was exposed to three tones (60 s, 65 dB white noise, separated by a 3-min interval). Animal images were captured twice per second using a camera, and the area (in pixels) in which the mouse moved was measured. Freezing was defined as a lack of movement such that fewer than 20 pixels changed between successive video frames for at least 2 s. The electrical intensity required to make the mouse jump was measured.

2.3. Immunoblot Analysis

Brain tissues were homogenized in cold lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 0.25% sodium dodecyl sulfate, 5 mM EDTA, and protease inhibitor cocktail (Sigma). Total protein concentrations in the supernatants were determined using a Bio-Rad protein assay kit (Hercules). The proteins were then analyzed by immunoblotting as follows. Protein samples were separated by reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% or 14% Tris-glycine

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