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Research Report

HO-1 induction in motor cortex and intestinal dysfunction in TDP-43 A315T transgenic mice

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ABSTRACT

TAR DNA-binding protein 43 (TDP-43) has been found to be related to the pathogenesis of amyotrophic lateral sclerosis (ALS). TDP-43 A315T transgenic mice develop degeneration of specific motor neurons, and accumulation of ubiquitinated proteins has been observed in the pyramidal cells of motor cortex of these mice. In this study, we found stress-responsive HO-1 induction and no autophagic alteration in motor cortex of TDP-43 A315T transgenic mice. Glial activation, especially astrocytic proliferation, occurred in cortical layer 5 and sub-meningeal region. Interestingly, we noticed that progressively thinned colon, swollen small intestine and reduced food intake, rather than severe muscle weakness, contributed to the death of TDP-43 A315T transgenic mice. Increased TDP-43 accumulation in the myenteric nerve plexus and increased thickness of muscular layer of colon were related to the intestinal dysfunction.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of upper and/or lower motor neurons. TAR DNA-binding protein 43 (TDP-43) is a RNA/DNA binding protein, containing two RNA-recognition motifs as well as a glycine-rich C-terminal sequence. TDP-43 has been implicated in the regulation of alternative splicing of messenger RNA, RNA stability and transcriptional control (Buratti and Baralle, 2008). In 2006, TDP-43 was identified as a pathological protein of ubiquitin-positive inclusions in frontotemporal lobar degeneration (FTLD) and ALS (Arai et al., 2006; Neumann et al., 2006). However, pathological TDP-43 protein was not found in familial ALS with Cu/Zn superoxide dismutase-1 (SOD1) mutation or SOD1-G93A high copy transgenic mice (Mackenzie et al., 2007).

Identification of TDP-43 Ala-315-Thr (A315T) mutation enriched the candidate genes in patients with familial ALS (Gitcho et al., 2008) and the link between altered TDP-43 function and neurodegeneration was provided. TDP-43 A315T transgenic mice were developed and aggregates of ubiquitinated proteins were observed in layer 5 pyramidal neurons in frontal cortex (Wegorzewska et al., 2009). However, no cytoplasmic TDP-43 aggregates were found in TDP-43 A315T mice. Autophagy is considered to play an important role in preventing the accumulation of abnormal proteins. Microtubule-associated protein 1 light chain 3 (LC3) and p62/SQSM1 both are important for autophagic process.

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LC3 is a well-characterized autophagosome membrane-linked protein (Kabeya et al., 2000), and p62 is a crucial adaptor between LC3-decorated autophagosomes and ubiquitinated protein aggregates (Komatsu et al., 2007). In the widely used SOD1-G93A transgenic mice, accumulation of SOD1-positive aggregates is one of the most characteristic changes in the affected regions (Sasaki et al., 2005). LC3II expression, which was known to be correlated with the extent of autophagosome formation, was increased in SOD1-G93A mice at symptomatic stage (Morimoto et al., 2007; Tian et al., 2011). At the same time, accumulation of p62 paralleled the increase of polyubiquitinated proteins and mutant SOD1 aggregates (Gal et al., 2007). However, to the best of our knowledge, whether or not autophagic alteration exists in the motor cortex of TDP-43 A315T transgenic mice has not been reported. Here, we also examined the status of glial activation by detecting astrocytic and microglial marker proteins, glial fibrillary acid protein (GFAP) and ionized calcium-binding adaptor molecule 1(Iba1), respectively. At the same time, antioxidative enzymes including Heme oxygenase-1 (HO-1), glutamate cysteine ligase catalytic subunit (GCLC) and glutathione synthetase (GSS) were investigated in motor cortex of TDP-43 A315T mice.

Our present findings together with a previous report (Wegorzewska et al., 2009) strongly suggest that muscle weakness and atrophy at end stage were not severe enough to cause death of TDP-43 A315T transgenic mice. Therefore, we also aimed to explore the lethal cause of TDP-43 A315T transgenic mice in this study.

Our present study showed that expression of stress-responsive HO-1 was induced in motor cortex of TDP-43 A315T mice, and increased GFAP and disturbed Iba1 immunostaining was observed in cortical layer 5 of brain in TDP-43 A315T mice. However, autophagy was not activated along with oxidative stress and reactive gliosis in motor cortex of TDP-43 A315T transgenic mice. Interestingly, we noticed progressively thinned colon, swollen small intestine and subsequent reduced food intake, which may be the cause of death in TDP-43 A315T mice. Our data also suggest that TDP-43 accumulation in the myenteric plexus may play a role in the pathological changes of intestinal tract in TDP-43 A315T transgenic mice.

2. Results

2.1. *Mutant TDP-43 expression in the central nervous system and autophagic examination in motor cortex of TDP-43 A315T transgenic mice*

TDP43 A315T transgenic mice were identified by PCR-based genotyping of tail DNA (Fig. 1A) and the life span of TDP-43 A315T transgenic male mice was 99.67±4.46 days. To investigate the regional difference in exogenous mutant TDP-43 expression in the central nervous system, we performed TDP-43 Western blot analysis. We found that TDP-43 A315T protein was relatively rich in the brain than in the spinal cord. Motor cortex was one of the regions that highly expressed TDP-43 transgene (Fig. 1B). Such regional difference was consistent with the relatively clear pathological changes in the cortex reported in a previous study

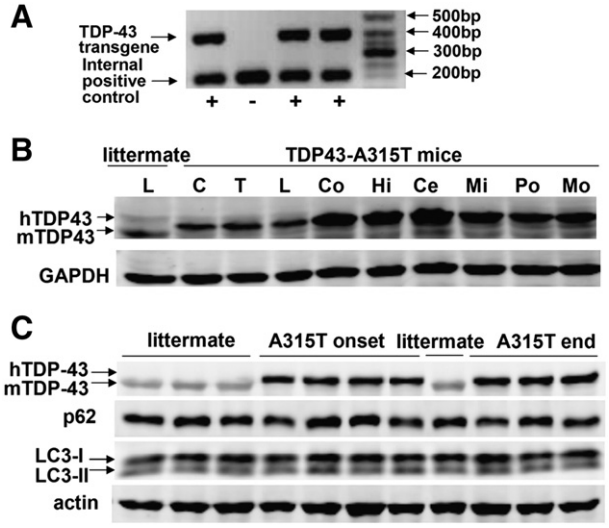

Fig. 1 – Identification of human TDP-43 transgenic mice, differential expression of TDP-43 mutant protein in the central nervous system, and examination of autophagy related markers in motor cortex. (A) PCR products of 400 bp and 200 bp indicate transgene and internal positive control, respectively. (B) Exogenous human TDP-43 (hTDP-43) has slightly larger molecular weight than endogenous mouse TDP-43 (mTDP-43). Exogenous mutant TDP-43 was relatively rich in motor cortex, hippocampus and cerebellum, compared with cervical, thoracic and lumbar spinal cord in each TDP-43 A315T transgenic mice at the end stage. (C) No significant difference in p62 and LC3 expression was found either at onset or end stage of TDP-43 A315 mice, as compared with control. C, cervical; T, thoracic; L, lumbar; Co, motor cortex; Hi, hippocampus; Ce, cerebellum; Mi, midbrain; Po, pons; Mo, medulla oblongata. hTDP-43, human TDP-43; mTDP-43, mouse TDP-43.

(Wegorzewska et al., 2009), which showed that ubiquitinated cytoplasmic aggregates were prominent features in layer 5 cortical neurons in TDP-43 A315T mice. Therefore, we further examined LC3B and p62 expression in motor cortex to find out whether autophagic alteration was involved in the pathogenesis of TDP-43 A315T-induced neurodegeneration. However, Western blot analysis of LC3B and p62 expression did not show any difference at either onset or end stage, compared with non-transgenic mice (Fig. 1C).

2.2. *Glial activation in motor cortex of TDP-43 A315T mice*

Although GFAP and Iba1 expressions examined by immunoblotting did not show clear induction in motor cortex of TDP-43 A315T mice, we found a certain degree of astrocytic activation in the layer 5 of cortex as well as in the submeningeal region, as analyzed by immunohistochemistry, which was consistent with a previous report (Wegorzewska et al., 2009). Iba1 immunostaining was not increased as much as that of GFAP, compared with controls, indicating that significant microglial proliferation did not occur. However, the distribution of Iba1-postive microglia was somewhat disturbed in layer 5 of motor cortex in TDP-43 A315T transgenic mice (Fig. 2).