Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Chronic cerebral ischemia induces redistribution and abnormal phosphorylation of transactivation-responsive DNA-binding protein-43 in mice

*Akihiro Shindo**, *Kenichiro Yata*, *Ryogen Sasaki*, *Hidekazu Tomimoto*

Department of Neurology, Graduate School of Medicine, Mie University, Japan

ARTICLE INFO

Article history:
Accepted 4 August 2013
Available online 14 August 2013

Keywords:
Chronic cerebral ischemia
TAR DNA-binding protein-43
Hippocampal sclerosis
Phosphorylation

ABSTRACT

Transactivation-responsive DNA-binding protein 43 (TDP-43) is closely involved in the pathogenesis of frontotemporal lobar degeneration. The native form, but not phosphorylated form, of TDP-43 has been reported to redistribute during acute neuronal injuries. Here, we examined whether the expression of phosphorylated TDP-43 was altered following chronic neuronal injury. C57BL/6 mice were subjected to sham operation or bilateral common carotid artery stenosis (BCAS) using microcoils, and changes in proteolytic cleavage, phosphorylation, and subcellular redistribution of TDP-43 were examined by immunoblotting and immunohistochemistry. We also monitored the expression of importin β, which is involved in the transport of TDP-43. Immunoblotting showed an increase in phosphorylated TDP-43 in the nuclear fraction after BCAS using microcoils. Moreover, immunoreactivity toward phosphorylated TDP-43 was observed in the neuronal cytoplasm in the cerebral cortex and hippocampus, and importin β levels decreased after the operation. Immunoreactivity toward phosphorylated TDP-43 was partly colocalized with immunoreactivity toward caspase 3 in the neuronal cytoplasm. These results suggested that chronic cerebral ischemia induced redistribution and abnormal phosphorylation of TDP-43, which may be triggered by downregulation of importin β and may partly result in neuronal death.

1. Introduction

Transactivation-responsive DNA-binding protein-43 (TDP-43) is a nuclear protein involved in transcriptional repression and alternative splicing. It was originally identified as a major component of ubiquitin-positive and tau-negative inclusions in the frontotemporal cortex and motor neurons during frontotemporal lobar degeneration (FTLD) with or without progranulin gene mutations and in amyotrophic lateral sclerosis (ALS) (Arai et al., 2006; Davidson et al., 2007;

Abbreviations: TDP-43, transactivation-responsive DNA-binding protein-43; p-TDP-43, phosphorylated transactivation-responsive DNA-binding protein-43; FTLD, frontotemporal lobar degeneration; ALS, amyotrophic lateral sclerosis; NLS, nuclear localization signal; PBS, phosphate-buffered saline; ss-DNA, single-stranded DNA

*Correspondence to: Department of Neurology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan. Fax: +81 59 231 5082.

E-mail addresses: a-shindo@clin.medic.mie-u.ac.jp (A. Shindo), ken-yata@mbk.nifty.com (K. Yata), ryogen@clin.medic.mie-u.ac.jp (R. Sasaki), tomimoto@clin.medic.mie-u.ac.jp (H. Tomimoto).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.08.007

Neumann et al., 2006). Several mutations in the *TARDBP* gene, which encodes TDP-43, were identified in sporadic or autosomal dominant familial ALS patients (Sreedharan et al., 2008). Therefore, since TDP-43 is thought to play an essential role in these disease conditions, they are collectively called TDP-43 proteinopathies. Consequently, TDP-43 proteinopathy has been defined as the abnormal accumulation of insoluble ubiquitinated TDP-43-containing inclusions, in which TDP-43 is abnormally phosphorylated and cleaved to generate a 25-kDa C-terminally truncated fragment lacking the consensus nuclear localization signal (NLS) at the N-terminus in the central nervous system (Arai et al., 2006; Neumann et al., 2006; Winton et al., 2008).

Currently, it is unclear how and why redistribution of TDP-43 is induced. Redistribution of TDP-43 is not a passive outcome during cytotoxic processes because TDP-43 is not found in the neuronal cytoplasm in human brains with anoxia or ischemia or after hippocampal resection for epilepsy (Lee et al., 2008). TDP-43 carries a bipartite NLS, and defects in this signal prevent nuclear transport (Winton et al., 2008). TDP-43 is a substrate of caspase-3, and caspase-cleaved TDP-43 exhibits a defective NLS, resulting in its exclusion from the nucleus (Zhang et al. 2007). Subsequently, TDP-43 proteins containing defective NLSs are readily phosphorylated and aggregate in the cytosol (Nonaka et al., 2009a).

These previous studies suggest that redistribution and abnormal phosphorylation of TDP-43 may not be specific to neurodegenerative processes. Indeed, abnormal accumulation of phosphorylated TDP-43 (p-TDP-43) has been observed in a broad range of neurodegenerative diseases encompassing not only FTLD and ALS, but also Guam ALS, Parkinsonism–dementia complex, and a subset of alpha-synucleinopathies and tauopathies, such as Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, and hippocampal sclerosis (Zarow et al., 2008). Furthermore, redistribution of TDP-43 in the neuronal cytoplasm has been reported experimentally in acute neuronal injuries after axonal ligation and cerebral ischemia (Kanazawa et al., 2011; Sato et al., 2009); however, abnormal phosphorylation is not detected in these cases.

Therefore, in the current study, we investigated the possible redistribution and abnormal phosphorylation of TDP-43 in chronic cerebral ischemia.

2. Results

To investigate changes in the expression and phosphorylation of TDP-43 in chronic cerebral ischemia, we performed immunohistochemical and immunoblot analyses of whole-cell extracts from chronic ischemic and sham-operated brains. All procedures for bilateral common carotid artery stenosis (BCAS) were completed within 15 min, except for an interval of 30 min between operating on the right and left common carotid arteries.

2.1. *Profile of white matter lesions*

As detailed in Table 1, white matter lesions were not detected in any regions of the brain in the sham-operated mice or in mice at 3 days and 1 week after BCAS. At 2 weeks after BCAS, white matter lesions were evaluated as grade 0 in 2 of 4 mice and grade 1 in the remaining 2 mice. At 3 weeks after BCAS, 1 of 4 mice was evaluated as grade 0, 2 were evaluated as grade 1, and 1 was evaluated as grade 2. Additionally, at 4 weeks after BCAS, 2 mice were evaluated as grade 1, 1 was evaluated as grade 2, and 1 was evaluated as grade 3.

2.2. *Subcellular localization and upregulation of TDP-43*

To investigate the subcellular localization of TDP-43 in chronic cerebral ischemia, we performed immunohistochemistry and immunoblot analyses. Neurons with immunoreactivities toward TDP-43 in both the nuclei and cytoplasm were detected after BCAS with numerical densities of 0.25 ± 0.50 (mean$\pm$SD) per 10 arbitrary fields, 4.00 ± 2.16 at 3 days, 6.00 ± 1.41 at 1 week, 7.75 ± 2.50 at 2 weeks, 7.50 ± 2.65 at 3 weeks, and 8.00 ± 2.16 at 4 weeks after the operation in the frontal cerebral cortex. In contrast, TDP-43 positivity was observed in neuronal nuclei in the sham group (Fig. 1A and B). To assess the effects of chronic cerebral ischemia on TDP-43 expression, we compared the severity of white matter lesions to the number of neurons with cytoplasmic TDP-43 and found a close correlation between these variables (Fig. 1C). In immunoblotting analysis, TDP-43 bands tended to decrease in the nuclear fraction after BCAS ($p=0.2334$, compared to the sham control and 1 week after BCAS), and bands in the cytoplasmic fraction increased between 1 and 2 weeks after BCAS (Fig. 1D and E).

2.3. *Abnormal phosphorylation of TDP-43*

To investigate whether TDP-43 was phosphorylated in chronic cerebral ischemia, we performed immunohistochemistry, confocal microscopy, and immunoblot analyses using antibodies specific for phosphoserines 409/410, that is, abnormal phosphorylation sites in TDP-43 proteinopathy (Hasegawa et al., 2008).

Table 1 – Profile of white matter lesions.

	Grade 0	Grade 1	Grade 2	Grade 3
Sham	4	0	0	0
After BCAS				
3 days	4	0	0	0
1 week	4	0	0	0
2 week	2	2	0	0
3 week	1	2	1	0
4 week	0	2	1	1