BRAIN RESEARCH 1249 (2009) 202–211

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: Implications for TDP-43 in the physiological response to neuronal injury

Katie Moisse[a,b], *Kathryn Volkening*[a], *Cheryl Leystra-Lantz*[a], *Ian Welch*[a,c], *Tracy Hill*[c], *Michael J. Strong*[a,b,d,*]

[a]*Molecular Brain Research Group, Robarts Research Institute, PO Box 5015, 100 Perth Drive, London ON, Canada N6A 5K8*
[b]*Department of Pathology, University of Western Ontario, 1151 Richmond Street, London ON, Canada N6A 5C1*
[c]*Department of Animal Care and Veterinary Services, University of Western Ontario, 1151 Richmond Street, London ON, Canada N6A 3K7*
[d]*Department of Clinical Neurological Sciences, University of Western Ontario, 1151 Richmond Street, London ON, Canada N6A 5A5*

ARTICLE INFO

Article history:
Accepted 4 October 2008
Available online 1 November 2008

Keywords:
Axotomy
TDP-43
Progranulin
Microglia
RNA granule
Amyotrophic lateral sclerosis

ABSTRACT

We have performed sciatic axotomies in adult C57BL/6 mice and observed TDP-43 and progranulin (PGRN) expression patterns over 28 days. TDP-43 expression was markedly upregulated in axotomized motor neurons, with prominent cytosolic immunoreactivity becoming maximal by post-injury day 7 and returning to baseline levels by post-injury day 28. Increased TDP-43 expression was confirmed by western blot. TDP-43 mRNA expression was also increased. This was inversely correlated with neuronal PGRN expression which was clearly reduced by day 7 with a return to baseline by post-injury day 28. In contrast, microglial PGRN expression was dramatically increased, and correlated with the inflammatory response to axotomy. Cytosolic TDP-43 colocalized with Staufen and TIA-1, markers for RNA transport and stress granules respectively. We did not observe colocalization of TDP-43 or PGRN with degradative granules (P-bodies) or activated caspase 3. These results indicate that TDP-43 expression is altered in response to neuronal injury and that normal expression is restored following recovery. These findings suggest that the upregulation of TDP-43 expression with prominent cytosolic localization in motor neurons injured by degenerative processes such as ALS may actually represent an appropriate response to neuronal injury.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease characterized by motor neuron degeneration. The disease cause remains elusive, although several contributing factors have been identified including mutant superoxide dismutase 1 (mutant SOD1) expression, TAR DNA binding protein (TDP-43) aggregation, progranulin (PGRN) mutations, aberrant inflammatory processes, and protein aggregation (Arai et al., 2006; Moisse and Strong, 2006; Strong, 1999; Neumann et al., 2006; Rosen et al., 1993; Strong et al., 2005; Eriksen and Mackenzie, 2008). It is likely that all of these factors contribute to the disease state and its progression (Strong, 2001).

* *Corresponding author*. Rm C7-120, UH-LHSC, 339 Windermere Road, London, Ontario, Canada N6A 5A5. Fax: +1 519 663 3609.
E-mail address: mstrong@uwo.ca (M.J. Strong).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.10.021

TDP-43 has been recently identified as a component of ubiquitinated inclusions seen in degenerating motor neurons in ALS (Arai et al., 2006; Neumann et al., 2006). It is a ubiquitously expressed 414-amino acid protein with DNA, RNA, and protein binding capabilities (Buratti and Baralle, 2008). However, the normal function of TDP-43 is unclear. In ALS, the expression of TDP-43 is upregulated by approximately 1.5 fold (Geser et al., 2008; Mishra et al., 2007) and in contrast to its normal nuclear localization, becomes cytosolic. TDP-43 immunoreactive skeins, filamentous cytosolic inclusions that are considered to be pathological protein aggregates, are often associated with ubiquitin suggesting that they are being targeted for degradation (Strong et al., 2007). In addition, TDP-43 isolated from ALS tissues is aberrantly phosphorylated, tends to form high molecular weight aggregates, and is associated with 25 kDa and 35 kDa proteolytic fragments (Arai et al., 2006; Neumann et al., 2006) although recent evidence suggests that this may represent some degree of post-mortem artifact (Inukai et al., 2008). Of particular interest, TDP-43 is a component of RNA granules isolated from rat brain (Elvira et al., 2006), suggesting that it may play a critical role in mRNA trafficking.

The activity of TDP-43 also appears to be integrally related to that of progranulin (PGRN). Mutations in the gene encoding the multifunctional secreted growth factor PGRN that lead to decreased protein expression levels have been identified in patients with ALS with frontotemporal dementia (ALS-FTD) (Cruts et al., 2006a,b; Cruts and Van, 2008; Schymick et al., 2007; Baker et al., 2006). PGRN expression within the central nervous system (CNS) is restricted to neurons and microglia (Mackenzie et al., 2006; Mukherjee et al., 2006; Ahmed et al., 2007; Baker et al., 2006) and is upregulated in response to infection or injury in the CNS (Baker et al., 2006; Eriksen and Mackenzie, 2008). Interestingly, knockdown of neuronal PGRN expression *in vitro* using small interfering RNA results in TDP-43 abnormalities reminiscent of those seen in ALS (Zhang et al., 2007), suggesting that abnormal cleavage and mislocalization of TDP-43 mediated by loss of PGRN function may contribute to neurodegeneration. However, the involvement of PGRN in the cleavage of TDP-43 to c-terminal pathological fragments has been disputed by work performed in mammalian culture (Shankaran et al., 2008). Thus the role of PGRN and TDP-43, including any role that the c-terminal fragments may have in neurodegeneration, remains unclear.

Degenerating motor neurons in ALS are also characterized by the presence of neurofilament (NF) aggregates (Strong et al., 2005). These aggregates are associated with a marked suppression of the low molecular weight NF subunit (NFL) mRNA steady state levels relative to those of either the middle (NFM) or high (NFH) molecular weight NF mRNA levels (Wong et al., 2000; Menzies et al., 2002; Bergeron et al., 1994). This alteration in the stoichiometry of expression of the NF subunit mRNAs is sufficient to induce NF aggregates in transgenic mice (Lariviere and Julien, 2004) and appears to be related to alterations in the expression of key NF mRNA binding proteins (Ge et al., 2007). It is of note therefore that TDP-43 can bind to, and stabilize, NFL mRNA suggesting that it has a role in the regulation of the cytoskeletal protein synthesis (Strong et al., 2007).

In order to further examine this process, we have employed a model of acute neuronal injury. In contrast to the dramatic cell loss associated with axotomy of central neuron populations (Lieberman, 1971; Price and Porter, 1972), axotomized peripheral neurons recover (Watson, 1974) in a well characterized series of complex biochemical alterations leading to the synthesis of cytoskeletal proteins required for axonal repair (Price and Porter, 1972). Within the same recovery period, there is an associated recovery of function (Price and Porter, 1972). Therefore the axotomy model permits both behavioural observation as well as histological correlates of neuronal repair.

Immediately following axotomy there is a dramatic decrease in NF mRNA and protein (Tetzlaff et al., 1988) followed by partial recovery within 28 days after axotomy (Goldstein et al., 1988). Because TDP-43 binds to and stabilizes NF mRNA (Strong et al., 2007), as well as β-actin mRNA (Wang et al., 2008), we were interested in determining whether TDP-43 expression is altered following neuronal injury. Similar to the axotomy model, NFL mRNA levels are reduced in ventral horn motor neurons undergoing degeneration due to ALS (Bergeron et al., 1994).

In these studies, we show that there is an increase in expression and relocalization of TDP-43 from the nucleus to the cytosol following peripheral axotomy which is associated with decreased neuronal PGRN expression. We also show that this is a reversible phenomenon. We have shown that TDP-43 may be involved in transporting and sequestering mRNA species necessary for the recovery from axotomy. These results are significant as they demonstrate that the increased expression and redistribution of nuclear TDP-43 to the cytosol is a normal physiological response to neuronal injury and that TDP-43 may in fact be functioning in an early role in neuronal repair.

2. Results

2.1. *Behavioural and histological correlates of neuronal injury following axotomy*

Although the axotomized mice exhibited left hindlimb paresis following either the proximal or distal axotomy, with the greatest deficit in those mice receiving proximal axotomy, by post-injury day 28, there was a significant recovery of function (Supplementary Fig. 1A). We confirmed the presence of axotomized motor neurons in ventral horns ipsilateral to the injury using fluorescent microscopy to detect fluorogold labelling throughout the 28 day duration of the study (Supplementary Fig. 1B). There were no fluorogold-positive motor neurons in the contralateral ventral motor neuron pool. In both the proximal and distal injury paradigms, an associated inflammatory response as exhibited by increased IBA-1 immunoreactivity in ventral horns ipsilateral to axotomy was observed. This response was maximal by day 7, and included an increased number of primed (intermediate between ramified and activated described in Moisse and Strong (2006)) and activated microglia. This response had resolved by post-injury day 28 (Supplementary Fig. 1C), and was not observed in the contralateral ventral motor neuron pool.