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Research Report

Survival motor neuron (SMN) protein in the spinal anterior horn cells of patients with sporadic amyotrophic lateral sclerosis

Yingshan Piao[a,b], Tomoyo Hashimoto[a,c], Sachiko Takahama[d], Akiyoshi Kakita[e], Takashi Komori[f], Takashi Morita[g], Hitoshi Takahashi[h], Toshio Mizutani[i], Kiyomitsu Oyanagi[a,j,*]

[a]Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan
[b]Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China
[c]Department of Safety and Health, Tokyo Gas Co. Ltd., Tokyo, Japan
[d]Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan
[e]Department of Pathological Neuroscience, Center for Bioresource-based Researches, Brain Research Institute, Niigata University, Niigata, Japan
[f]Department of Clinical Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan
[g]Department of Pathology, Shinrakuen Hospital, Niigata, Japan
[h]Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan
[i]Department of Laboratory Medicine, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan
[j]Department of Brain Disease Research, Shinshu University School of Medicine, Nagano, Japan

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving mainly the upper and lower motor neurons of adult humans. With regard to the pathomechanism of spinal anterior horn cell (AHC) degeneration in ALS, copy number abnormalities of the survival motor neuron (SMN) genes have been reported in sporadic (s) ALS. SMN protein is the protein responsible for the pathogenesis of spinal muscular atrophy (SMA), an autosomal recessive disease characterized by lower motor neuron loss and muscle atrophy. The disease is caused by deficiency of SMN protein induced by mutation of one of the SMA-associated genes, SMN1. To clarify the role of SMN protein in the degeneration of spinal AHCs in sALS, we examined the amount of cytoplasmic SMN protein in individual AHCs using cytofluorophotometry in 9 patients with sALS and 10 control subjects. It was found that: 1) SMN protein was present in the cytoplasm, nucleus and nucleolus of AHCs and in the nucleus of glial cells, 2) expression of SMN protein in AHCs was significantly associated with cell size in both sALS patients and controls, 3) expression of SMN protein per unit area in AHCs was similar in sALS patients and controls. These findings suggest that: 1) the amount of SMN protein in the cytoplasm of AHCs is strictly controlled in accordance with cell size, in both sALS patients and controls, 2) the amount of SMN protein in the AHCs of sALS patients may be reduced when the AHCs are atrophic, and 3) decrease of SMN

* *Corresponding author*. Department of Brain Disease Research, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. Fax: +81 263 37 3186.
E-mail address: k123ysm@shinshu-u.ac.jp (K. Oyanagi).

Abbreviations: ALS, amyotrophic lateral sclerosis; AHC, anterior horn cell; SMA, spinal muscular atrophy; SMN, survival motor neuron; IOD, integrated optical density; SOD1, superoxide dismutase; TDP-43, TAR DNA-binding protein

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protein in the AHCs of sALS patients may be a secondary, and not primary, phenomenon according to their sizes.

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by degeneration of lower motor neurons and muscle atrophy (Lefebvre et al., 1995). The majority of cases result from homozygous deletions or mutations of the survival motor neuron 1 (SMN1) gene. In humans, the SMN gene exists as two highly homologous copies, the telomeric copy (SMN1), which encodes the full-length protein, and the centromeric copy (SMN2), which encodes a truncated isoform (Lorson et al., 1999; Monani et al., 1999). The SMN1 gene is homozygously deleted in approximately 95% of SMA patients (Bussaglia et al., 1995; Velasco et al., 1996) and the SMN2 gene plays a role in modulating the severity of the phenotype (Anderson and Talbot, 2003). Furthermore, the level of SMN protein is markedly reduced in the spinal cords of patients with SMA (Coovert et al., 1997; Lefebvre et al., 1997). It has been considered that defective SMN protein disrupts normal cellular RNA metabolism, thus causing motor neuron degeneration (Kolb et al., 2007).

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving mainly the upper and lower motor neurons of adult humans. Among patients with ALS, 5–10% have the familial form, about 20% of such cases being associated with mutation in the Cu/Zn superoxide dismutase (SOD1) gene on chromosome 21 (Rosen et al., 1993). The remaining 90–95% of ALS cases are sporadic, and do not have an obvious family history (Schymick et al., 2007). The pathological hallmarks of sporadic (s) ALS are loss of motor neurons in the brain stem, spinal anterior horn cells (AHCs) and degeneration of the corticospinal tract, and the presence of Bunina bodies and ubiquitinated skein-like inclusions in the spinal cord (Kato et al., 2003; Piao et al., 2003; Tomonaga et al., 1978; Kato et al., 1989). Although the cause of sALS remains unknown in the great majority of cases, it is believed to be a multifactorial disease (Figlewicz and Orrell, 2003). With regard to the pathomechanism of AHC degeneration in ALS, Corcia et al. (2002a) reported that within three families ALS and SMA were concurrent. More recently, copy number abnormalities of the SMN genes have been reported in sALS. The frequency of patients with one or three abnormal copies of the SMN1 gene was reported to be significantly increased in sALS cases, although many control subjects show two copies (Corcia et al., 2002b, 2006), suggesting that the gene may be involved in sALS (Corcia et al., 2009). In addition, homozygous deletions of SMN2 are suspected to act as a susceptibility factor for ALS mainly involving lower motor neurons in adults (Moulard et al., 1998; Echaniz-Laguna et al., 2002; Kim et al., 2010), as well as a prognostic factor affecting survival time in patients with sALS (Veldink et al., 2001). Veldink et al. (2005) speculated that a smaller SMN protein might be expressed, based on a formula that takes into account the SMN1 and SMN2 gene copy numbers in sALS. Recently it has also been reported that reduction in the level of SMN protein in the spinal cord contributes to the pathogenesis of motor neuron death in transgenic SOD1 mice with G93A mutation, which has been used as a model of SOD1-linked ALS (Turner et al., 2009).

In addition, TAR DNA-binding protein (TDP-43) has recently been identified as the major disease protein of sALS (Neumann et al., 2006; Arai et al., 2006). Bose et al. (2008) have reported that TDP-43 overexpression enhances exon 7 inclusion during SMN2 pre-mRNA splicing, although global splicing in the cells remains to be investigated. Thus, SMN protein is becoming an increasing focus of attention in sALS research. However, no previous studies have examined the level of SMN protein in individual spinal AHCs in sALS patients.

In the present study, we examined the amount of SMN protein in individual spinal AHCs from sALS patients and controls using cytofluorophotometry of sections immunostained for SMN.

2. Results

2.1. *Expression of SMN protein in the lumbar spinal cord*

SMN protein was observed in both AHCs and glial cells in controls as well as sALS patients. SMN immunoreactivity was observed in the cytoplasm, nucleolus and nucleoplasm of the AHCs, the expression corresponding to that seen by immunohistochemistry using bright field and immunofluorescence microscopy (Figs. 1A (MO1 antibody) and 2E–H (H-195 antibody)). Omitting the primary antibody failed to show the positive immunostainability (Fig. 1B). In the cytoplasm, SMN protein was localized in the soma and proximal portion of neurites. In the nucleus, SMN immunolabeling was observed at the nuclear membrane, the nucleolus, and fine dot-like structures in the nucleoplasm, as reported previously (Liu and Dreyfuss, 1996). On the other hand, expression of SMN protein was also noted in glial cell nuclei in the spinal cord in the present study (Figs. 1A and 2E and H).

As in the controls (Fig. 2A and E), SMN protein expression was observed in the cytoplasm, nucleolus, nucleoplasm and nuclear membrane of chromatolytic (Fig. 2C and G) and shrunken (Fig. 2D and H) AHCs, and also in normal-looking AHCs (Fig. 2B and F) in ALS patients.

2.2. *Amount of cytoplasmic SMN protein in AHCs of the lumbar spinal cord*

We examined the amount of cytoplasmic SMN protein using immunofluorophotometry. We plotted in Fig. 3 the integrated optical density (IOD) of SMN protein against the area of cytoplasm in the AHCs of the lumbar spinal cord based on data for 129 AHCs from sALS patients and 144 AHCs from controls. The IOD of the SMN protein in each AHC showed a cell size-