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Differences in protein quality control correlate with phenotype variability in 2 mouse models of familial amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a disease of variable severity in terms of speed of progression of the disease course. We found a similar variability in disease onset and progression of 2 familial ALS mouse strains, despite the fact that they carry the same transgene copy number and express the same amount of mutant SOD1G93A messenger RNA and protein in the central nervous system. Comparative analysis of 2 SOD1G93A mouse strains highlights differences associated with the disease severity that are unrelated to the degree of motor neuron loss but that appear to promote early dysfunction of these cells linked to protein aggregation. Features of fast progressing phenotype are (1) abundant protein aggregates containing mutant SOD1 and multiple chaperones; (2) low basal expression of the chaperone alpha-B-crystallin (CRYAB) and β5 subunits of proteasome; and (3) downregulation of proteasome subunit expression at disease onset. In contrast, high levels of functional chaperones such as cyclophillin-A and CRYAB, combined with delayed alteration of expression of proteasome subunits and the sequestration of TDP43 into aggregates, are features associated with a more slowly progressing pathology. These data support the hypothesis that impairment of protein homeostasis caused by low-soluble chaperone levels, together with malfunction of the proteasome degradation machinery, contributes to accelerate motor neuron dysfunction and progression of disease symptoms. Therefore, modulating the activity of these systems could represent a rational therapeutic strategy for slowing down disease progression in SOD1-related ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by adult-onset progressive loss of upper motor neurons in the cortex and brainstem and lower motor neurons in the ventral horns of the spinal cord. Although only approximately 5%–10% of ALS cases are inherited (familial ALS [fALS]), sporadic and familial forms share similar clinical and pathologic features, suggesting the involvement of common mechanisms of degeneration. ALS is a heterogenous disease in terms of age of symptom onset and disease duration, which is usually less than 5 years from diagnosis, although 20% of patients

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live longer and 10% of patients live ≥10 years (Beghi et al., 2011). The biological basis of such differences in the speed of disease progression is not understood. Even in families with specific gene mutations, affected members may manifest clinical heterogeneity in terms of age, site of onset, and disease progression rate. These features suggest the presence of gene modifiers and pathways that specifically affect the disease phenotype (Camu et al., 1999). For example, mutations in the Cu-Zn superoxide dismutase 1 (*SOD1*) gene (>140 discovered, http://alsod.iop.kcl.ac.uk), which are responsible for 1/5th of fALS cases (Andersen et al., 2003), are characterized by a considerable interfamilial and intrafamilial variabilities in the phenotype (Cudkowicz et al., 1997). These *SOD1* gene mutations, the majority of which are missense substitutions, result in a toxic gain of function of the enzyme (Valentine et al., 2005). Some mutations causing a high propensity for mutant *SOD1* to form aggregates, for example A4V, have been associated with a faster disease course (Prudencio et al., 2009; Wang et al., 2008). Thus, factors beyond the causative gene mutation can modify the disease course and may contribute to the heterogeneous phenotype of ALS patients. Identifying these putative modifier factors might eventually lead to discovery of prognostic markers of disease and allowing the design of more specific therapeutic strategies for individual or homogeneous groups of patients.

Transgenic mice overexpressing various human SOD1 mutations develop severe and progressive motor neuron degeneration that leads to muscular weakness and paralysis culminating in death (Bendotti and Carri, 2004). In particular, one of the most used and best characterized mouse models is the mutant hSOD1G93A (B6SJL-TgSOD1G93A-1Gur) expressing ∼20 copies of mutant human *SOD1* with a Gly93Ala substitution (Rosen et al., 1993). These mice recapitulate several aspects of the clinical profile of human patients, thus representing a useful model of the pathology (Gurney et al., 1994). Disappointingly, neuroprotective therapies reported to positively affect the disease course in this mouse model have translated poorly into beneficial effects in ALS clinical trials (Benatar, 2007; Scott et al., 2008).

This failed translation can be attributed to several different factors as reported in the guidelines for preclinical research in ALS and/or motor neuron disease (Ludolph et al., 2007, 2010) including the phenotypic variability between individuals. As in human ALS, the mutant SOD1 mouse models may vary in terms of age of onset, disease progression, and certain histopathologic features (Kunst et al., 2000; Pan et al., 2012) even if they carry the same mutation, for example, SOD1G93A. Indeed, it has been reported by several groups, including the Jackson Laboratory, that the disease duration and survival of SOD1G93A mice are affected by their genetic background (Heiman-Patterson et al., 2005, 2011; Mancuso et al., 2012). Because all the ALS mouse strains reported in these studies express the same number of copies of the human SOD1 transgene, the different phenotype could be because of the modifier genes. These may even influence the expression level of the mutant SOD1 gene products in the vulnerable tissues and/or alter the pathogenic molecular and cellular mechanisms triggered by the presence of mutant SOD1.

Recently, we also observed that transgenic SOD1G93A mice under different genetic backgrounds, namely C57BL/6J and 129S2/Sv, showed a clinical phenotype that differs consistently in terms of the rate of disease progression and life span. In addition to the phenotype variation, these 2 SOD1G93A mouse strains responded differently to treatment with lithium and omega 3 (Pizzasegola et al., 2009; Yip et al., 2013). Therefore, in this study, we aimed to investigate the potential molecular mechanisms responsible for the phenotypic differences between these 2 SOD1G93A mouse strains and to analyze the histopathologic and biochemical profiles of the central nervous system during disease progression. In particular, because the 2 strains exhibit remarkable differences in the levels of insoluble protein in the spinal cord at comparable stages of the disease, we investigated thoroughly this phenomenon through a comparative analysis of the mechanisms controlling protein degradation including chaperone protein expression, the ubiquitin-proteasome system, and the autophagy-lysosomal pathway.

2. Materials and methods

2.1. Mouse models

Mice were maintained at a temperature of 21 ± 1 °C with a relative humidity of 55% ± 10% and 12 hours of light. Food (standard pellets) and water were supplied ad libitum. All the procedures involving animals and their care carried out at the Mario Negri Institute were conducted as described by the institutional guidelines that are in accordance with national (D.L. no. 116, G.U. suppl. 40, February 18, 1992, no. 8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358, December 12,1987; National Institutes of Health Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996). Transgenic SOD1G93A mice expressing ∼20 copies of mutant human SOD1 with a Gly93Ala substitution (B6SJL-TgSOD1G93A-1Gur) were originally obtained from Jackson Laboratories and maintained on a C57BL/6JOlaHsd (C57) genetic background at Harlan Italy S.R.L., Bresso, Milan, Italy. From the crossbreeding of C57BL/6JOlaHsd (C57G93A) with 129S2/SvHsd (129Sv) for >15 generations, we obtained SOD1G93A mice on the homogenous background 129SSv (129SvG93A). Female transgenic C57G93A or 129SvG93A and corresponding nontransgenic (Ntg) female littermates were used in this study.

For biochemical and immunohistochemical analyses, SOD1G93A mice of both strains were euthanized with ketamine (75 mg/kg) and medetomidine (1 mg/kg) at the presymptomatic (PS), symptom onset (onset), and symptomatic (symp) stages of disease corresponding to the C57G93A mice at 12, 18, and 22 weeks, respectively, and to the 129SvG93A mice at 10, 14, and 16 weeks of age, respectively. The corresponding age-matched nontransgenic mice were used as controls.

2.2. Copy number analysis

Mice positive for transgene expression were then subjected to copy number analysis by quantitative polymerase chain reaction (PCR) according to the protocol described by Mead et al. (2011). Briefly, 2 ng DNA was mixed in 1× SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 0.3 μM human SOD1 primers (forward [F′] 59-CCAAGGAGCAGATCATAGGGC-3; reverse [R′] 59-AGAGCATTGGAGAAGGCAGG-39) in a total volume of 25 μL. After an initial denaturation at 95 °C for 10 minutes, DNA was amplified by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute on a 7300 Real-Time PCR System (Applied Biosystems).

2.3. Analysis of disease progression and survival length

Analysis of motor dysfunction was performed by measuring the deficit in grip strength and rotarod performance as reported previously (Pizzasegola et al., 2009). The onset of motor symptoms was defined as the point when the mice showed the first impairment in grip strength. Disease end stage was defined when the animals were unable to right themselves within 10 seconds after being placed on each side. This time point was considered as the index of